HISTOMORPHOLOGICAL EVALUATION OF ESOPHAGUS IN CASES OF GASTRO ESOPHAGEAL REFLUX DISEASE AND ITS ASSOCIATION WITH GASTRIC H. PYLORI INFECTION

DISSERTATION SUBMITTED FOR

M.D. (PATHOLOGY)

THE TAMILNADU DR.M.G.R.MEDICAL UNIVERSITY,

CHENNAI, TAMILNADU

SEPTEMBER - 2009

CERTIFICATE

This is to certify that the dissertation entitled, **"Histomorphological Evaluation of Esophagus in Cases of Gastro Esophageal Reflux Disease and its association with Gastric H. Pylori Infection",** by Dr.A.R. Radhika, Post graduate in Pathology (2006-2009) is a bonafide research work carried out under our direct supervision and guidance and is submitted to The Tamilnadu Dr.M.G.R. Medical University, Chennai, for M.D. Degree Examination in Pathology, Branch III, to be held in **September 2009.** This work has not previously formed the basis for the award of any degree or diploma

Date:

Place:

ETHICAL CLEARANCE CERTIFICATE

The **Ethical Committee of Tirunelveli Medical College and hospital has approved the study,**

“Histomorphological Evaluationof Esophagus in Cases of Gastro Esophageal Reflux Disease and its association with Gastric H. Pylori Infection”

submitted by Dr. A.R. Radhika, Post graduate in Pathology, Tirunelveli Medical College, following the regulations and guidelines.

Date:

Place:

CHAIRMAN

FOR ETHICAL COMMITTEE

Office seal

ACKNOWLEDGEMENT

It is with deep sense of gratitude that I acknowledge my indebtness to **Dr. Sithi Athia Munuvarah**. M.D, Professor and Head of Department of Pathology and **Dr. V. Paramasivam**. MD (Path), MD(FM) , former professor and HOD of Pathology, for suggesting this work for dissertation and their unstinted guidance which have been the motive force in bringing worth this piece of work.

I acknowledge my thanks to Dr. K. **Swaminathan** MD., **Dr. K. ShanthaRaman** MD, **Dr. S. Vallimanalan** MD., **Dr. Arasi Rajesh** MD, **Dr. J. Suresh Durai** MD, Readers in Department of Pathology , for their encouragement, guidance and timely advice.

I would like to place on record my thanks to **Dr. N. Jeyabalan** MD, and **Dr. S. Vasuki** Assitant professors in Department of Pathology for their help in carrying out this study.

It is my duty to thank **Dr. G. BadariNarayanan** MD. DM consultant Gastroenterologist for allowing me to use his valuable clinical material.

I thank all the technical assistants for their needy help. I honour the help and assistance rendered by my senior and junior colleagues and I thank all the patients involved in this study for their co-operation.

And, of course, I'm most indebted to my Beloved Family, my friends and THE ALMIGHTY.

CONTENTS

INTRODUCTION

In the later half of the twentieth century, gastro-esophageal reflux disease [GERD] emerged as the most common upper gastrointestinal disease of the western world. This disease is a major contributor to the rise in the war of endoscopy and acid suppression therapy.

Epidemiolgic studies suggest a 3% to 4% prevalence of GERD in the general population with the preponderance of individuals having mild or moderate disease. Reflux esophagitis results from the action of peptic juice on the esophageal mucosa. Reflux esophagitis can occur in any age group but is most common in the middle aged persons. Risk factors include hiatal -hernia, excessive vomitting and peptic ulcer disease. The use of Non - steroidal antiinflammatory agents, alcohol abuse, cigarette smoking, diabetes, systemic sclerosis and pemphigus are also associated with gastro -esophageal reflux.

Barrett's esophagus, defined as the presence of the columnar metaplastic epithelium in the distal esophagus over a length of more than 2 to 3cm is usually considered to be a complication of long standing gastro-esophageal reflux disease [GERD] and is one of the major manifestations of GERD.

Helicobacter pylori is a gram-negative, microaerophilic bacterium that inhabits various areas of the stomach and duodenum. It causes a chronic low-level inflammation of the stomach lining and is strongly linked to the development of duodenal and gastric ulcers and stomach cancer. Over 80% of individuals infected with the bacterium are asymptomatic.

Helicobacter pylori has acquired great importance during the last two decades, after being recognized as an important pathogen that infects a great portion of the

human population. Helicobacter pylori is of major concern today because of its causal relationship with gastroduodenal diseases. The bacteria are prevalent worldwide and more than half of the world's population are infected with H. pylori.

Recently, it has been observed that gastric colonization with H.pylori may also have beneficial effects for the human host. In this respect, the interest is in particular going to the potential preventive effect of H.pylori colonization on the development of gastro-esophageal reflux disease (GERD) and its complications such as Barrett's esophagus and adenocarcinoma of the distal esophagus. If so, this will have a major impact on issues such as screening and treatment of H.pylori infections.

By now, the potential role of H. Pylori in the development of GERD is a key issue in the treatment of patients with upper gastrointestinal disorders.

GERD patients with concomitant H.pylori infection showed more severe gastritis in the antrum than in other parts of the stomach, such as corpus, fundus and cardia.

Apart from a lower prevalence of GERD among H.pylori-positives, some also reported that if GERD is present in H.pylori-positive subjects, it may be less severe.

This interesting note made us think to evaluate the various histomorphological changes seen in esophagus in cases of GERD and to correlate the findings with the status of gastric H pylori colonization in those cases.

AIM & OBJECTIVES

Aim:

To study the histomorphological changes of esophagus in cases of GERD and its association with gastric H.pylori infection.

Objectives:

1. To evaluate the histomorphological profiles of esophagus in cases of GERD.
2. To evaluate the presence of gastric H.pylori infection in these lesions.
3. To correlate the histomorphological changes in the esophagus with the gastric H pylori status.

REVIEW OF LITERATURE

Epidemiolgic studies suggest a 3% to 4% prevalence of Gastroesophageal reflux disease (GERD) in the general population with the preponderance of individuals having mild or moderate disease. Reflux esophagitis results from the action of peptic juice on the esophageal mucosa. Reflux esophagitis occurs in any age group but is most common in the middle aged persons. Risk factors include hiatal -hernia, excessive vomiting and peptic ulcer disease. The use of Non - steroidal anti inflammatory agents, alcohol abuse, cigarette smoking, diabetes, systemic sclerosis and pemphigus are also associated with gastro -esophageal reflux. Acid or alkaline reflux may be caused by an incompetent Lower Esophageal sphincter (LES) or altered esophagel motility. Incompetence of the LES is usually idiopathic, but it may also be attributable to alcohol ingestion, Cigarette smoking, or the use of therapeutic drugs (such as estrogens). The LES tone is also lowered during pregnancy and naso gastric intubations.

Gastro-esophageal reflux disease affects patients of all ages, even in children and infants. It is equally present among men and women, but there is a slight male predominance of esophagitis and Barrett's esephagus. Both genetic factors and environmental factors play a role in predisposition to GERD. Sonnenberg and Serag (1999).

The predisposing conditions to GERD include smoking, increased intra-abdominal or intra-gastric pressure, including pregnancy, ascites, obesity and delayed gastric emptying. Motility disorders including diabetes, alcoholic neuropathies, achalasia and scleroderma also predispose to GERD. It also follows surgical procedures involving the lower end of esophagus such as esophagogastrostomy, Miller LS and Vinayek et al (1990). GER in infants and children complicates

congenital esophageal or gastric abnormalities.GER also associates with cystic fibrosis. (Button BM, Roberts.S, et al) (2005).

GERD is a multifactorial disorder. Most patients with GERD have a lower mean esophageal sphincter resting pressure. This allows acid to reflux into the esophagus, leading to the development of esophagitis. The inflammation further impairs the LES pressure, increasing acid exposure to the esophagus, Biancani P, and Billetta, et al (1992). The nature and amount of refluxed material and the length of the time it remains in contact with the esophageal mucosa, as well as the number of reflux episodes, determine whether GERD develops.

GERD results from reflux of both acid and alkaline secretions. Acid when combined with pepsin or bile acids causes more severe damage. Fiorucci S, Santucci L, et al (1992).

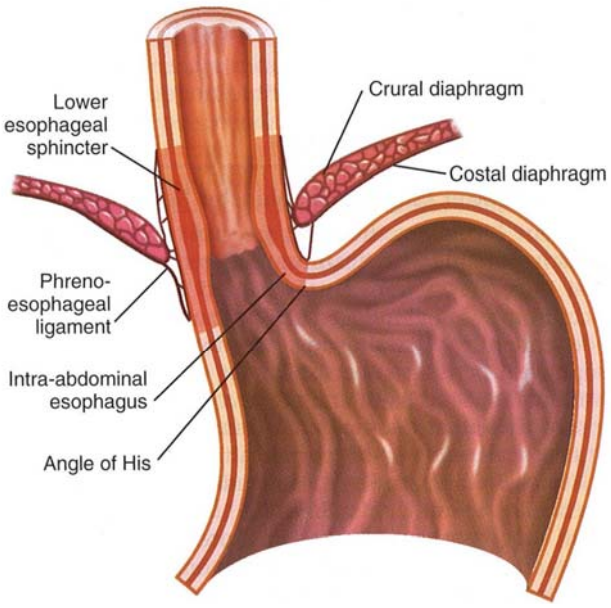
The patients usually present with disease symptoms including heartburn, regurgitation, bitter tasting fluids in the mouth, dysphagia, odynophagia, nausea, vomiting, hiccups, angina like chest and hoarseness. It is especially common in preterm infants. Complications peak between the age of 50 and 70 years. Nebel OT and Fornes MF et al (1976). The severest complication is carcinoma developing in the setting of Barrett's esophagus.

GERD is the failure of the normal anti-reflux barrier to protect against frequent and abnormal amounts of gastro-esophageal reflux. GERD is complex, resulting from an imbalance between defensive factors protecting the esophagus (anti-reflux barriers, esophageal acid clearance, tissue resistance) and aggressive factors from the stomach (gastric acidity, volume & duodenal contents).

The anti reflux barrier is an anatomically complex region including the intrinsic Lower esophageal sphincter, diaphragmatic crura, the intra-abdominal

location of the Lower esophageal sphincter, the phrenoesophageal ligaments and the acute angle of HIS.

ANATOMY OF THE GASTRO ESOPHAGEAL JUNCTION ILLUSTRATING THE MAJOR ELEMENTS OF THE ANTI-REFLUX BARRIER.



The Lower esophageal sphincter, involves the distal 3 to 4 cms of the esophagus and at rest is tonically contracted. Liebermann – Meffert D, Alogower M, et al (1979). Lower esophageal sphincter is the major component of the anti-reflux barrier. Sloan S, Rade maker AW et al (1992). The proximal Lower esophageal sphincter border is normally 1.5 to 2 cms above the squamo-columnar junction, whereas the distal segment, about 2 cm in length, lies within the abdominal cavity. This location maintains gastro-esophageal competence during the intra-abdominal pressure events. The Lower esophageal sphincter, maintains a high pressure zone by the intrinsic tone of its muscle and by cholinergic excitatory neurons. Dodds WJ, and

Dent J et al (1981). There is considerable diurnal variation in basal Lower esophageal sphincter pressure. It is lowest after meals and highest at night.

The mechanisms of reflux are

1. Transient lower esophageal sphincter relaxations.
2. Swallow induced lower esophageal sphincter relaxations
3. Hypotensive lower esophageal sphincter pressure.

Transient lower esophageal sphincter relaxations are the most frequent mechanism for reflux in patients with healthy sphincter pressures. Transient Lower esophageal sphincter relaxations are independent of swallowing, are not accompanied by esophageal peristalsis, persist longer (>10 seconds) and are accompanied by inhibition of the crural diaphragm. Holloway RH, penagini R et al (1995). The dominant stimulus for transient Lower esophageal sphincter relaxations is distention of the proximal stomach by either food or gas. Mittal RK, Holloway RH et al (1995). Holloway RH, kocyan p, et al (1991).

Swallow induced lower esophageal sphincter relaxation:

Almost 5 to 10 % of reflux episodes occur during swallow – induced Lower esophageal sphincter relaxations. Most episodes are associated with defective or incomplete peristalsis. Mittal RK, Mc callum RW: 1987. Reflux during swallow induced Lower esophageal sphincter relaxation's is more common with a hiatal hernia, this may be due to the lower compliance of the esophagogastric junction in hernia patients. Mittal RK and Lange RC et al (1987). Pandofino JE and shi G et al (2003).

The second tier against reflux damage is the "oseophageal acid clearance". There are two separate processes which are (i) Volume clearance: i.e the actual

removal of the reflux material from the esophagus (ii) Acid clearance; i.e the restoration of normal esophageal pH following acid exposure through titration with base from saliva & esophageal gland secretions.

Esophageal peristalsis clears acid volume in both upright & supine positions. Helm and colleagus showed that one or two primary peristaltic contractions will completely clear 15 ml fluid bolus from the esophagus, Helm JF and dodds WS et al (1984). Primary peristalsis is elicited by swallowing. Secondary peristalsis initiated by esophageal distension from acid reflux, is much less effective in clearing the refluxate. Peristaltic dysfunction and hypotensive peristaltic contractions increases in frequency with severity of oesophagitis. Kahrilas and colleagues found that the prevalence of peristaltic dysfunction rose from 25% in individuals with mild esophagitis to more than 50% in patiants with severe esophagitis. Kahrilas PS and dodds WJ et al (1986).

Saliva is the second essential factor required for normal oesophageal acid clearance. Saliva is a weak base with a pH of 6.4 to 7.8 compared to gastric acid. Helm JF and Hogan WJ et al (1987). Hence saliva is ineffective in neutralizing large acid volume, it easily neutralizes the small amount of acid remaining in the oesophagus after severe peristaltic contractions. Helm JF and Doods WJ etal (1984).

Modulation of salivation may contribute to GERD. Decreased salivation during sleep is the reason that nocturnal reflux episodes are associated with markedly prolonged acid clearance. Orr WC and Robinson MG et al (1987). Xerostomia patients and cigarette smokers have a prolonged esophageal acid clearance time due to hyposalivation. Korstem MA and Rosman As, et al (1991). In addition to saliva, the aqueous bicarbonate rich secretions of the esophageal sub-mucosal glands dilute and neutralize the residual esophageal acid. Meyers RL and orlando RC, et al (1992).

Only a few subjects experience symptomatic GER and even fewer persons suffer GERD, due to a phenomenon of esophageal defense known as tissue resistance. Tissue resistance can be subdivided into pre-epithelial, epithelial, post-epithelial factors which act together to minimize mucosal damage from the noxious gastric refluxate. Orlando RC 1994.

The pre-epithelial defense in the esophagus is poorly developed. The epithelial defense consists of both structural and functional components. Structural components include the cell membranes and inter-cellular junctional complexes of the esophageal mucosa. The functional components of tissue resistance include the ability of the esophageal epithelium to buffer and extrude hydrogen ions. Intracellular buffering is accomplished by negatively charged phosphates and proteins as well as bicarbonate ions. The post-epithelial defense is provided by the esophageal blood supply. Blood supply delivers oxygen, nutrients, bicarbonates and removes H^+ ions and CO2, there by maintaining normal tissue acid –base balance. Hollowarth ME and smith M et al (1986).

Gastric factors (volume and ingredients in the gastric refluxate) are potentially important in the production of reflux esophagitis. Gastric acidity determines the degree of potential mucosal damage of the refluxate. Acid and activated pepsin are the key ingredients of the gastric refluxate producing esophagitis. Acid alone causes protein denaturation. Acid when combined with even small amounts of pepsin, disrupts the mucosal barrier, resulting in increased H^+ permeability, histologic changes and hemorrhage, Orlando RC and Bryson JC et al (1984).

Along with acid and pepsin, duodenal contents may be injurious to the esophageal mucosa. Duodeno-gastric reflux into esophagus predisposes to

complications of GERD. Attwood SEA and DeMeester TR, et al (1978). Pellegrini CA and Wernly JA, et al (1978).

Delayed gastric emptying is a major factor contributing to GERD in some groups such as diabetic patients with autonomic peripheral neuropathy.

Esophageal lesions in Gastroesophageal reflux disease (GERD):

Esophageal biopsies are used for evaluation of GERD. Microscopic changes of reflux may occur even when the mucosa endoscopically appears normal. The changes of basal cell hyperplasia and increased height of the retepegs both representing increased epithelial turn over of the squamous mucosa.

Biopsies are taken in the area just distal to the Z line to detect carditis, just proximal to the Z line to detect the hyperplastic changes that are more predictive of the presence of GERD than more distally derived biopsies.

There are generally four stages in the disease progression in cases of reflux esophagitis:

(a). Acute (necrosis, inflammation & granulation tissue formation)

(b). Repair (Basal cell hyperplasia and elongation of the papillae)

(c). Chronic (fibrosis and formation of Barrett's esophagus)

(d). Complications (Dysplasia and adenocarcinoma)

HISTOLOGIC FEATURES IN GASTROESOPHAGEAL REFLUX DISEASE (GERD):

- Epithelial hyperplasia
- Basal zone hyperplasia.
- Papillary elongation.
- Ballooned squamous cells
- Vascular dilation.
- Intra-epithelial eosinophills

- Intra-epithelial Lymphocytes.
- Neutrophil infiltration
- Mucosal ulceration / erosion

Endoscopic features:

Approximately one third of the patients with chronic gastroesophageal reflux disease symptoms are endoscopically normal. Areas of patchy erythema and red streaks are seen in initial stages. Later, erosions and ulcers develop. Esophagus appears friable and diffusely reddened and hemorrhagic.

ENDOSCOPIC GRADING SYSTEMS FOR OESOPHAGITIS

SAVARY MILLER CLASSIFICATION

Grade 0	:	Not applicable
Grade I	:	Single, erosive or exudative lesion on one longitudinal fold
Grade II	:	Multiple erosions on more than one longitudinal fold
Grade III	:	Circumferential erosions
Grade IV	:	Ulcers, strictures or short oesophagus, isolated or associated with grades I to III
Grade V	:	Barrett's oesophagus + Grade I to III

Los Angeles Classification:

GRADE A : One or more mucosal breaks confined to fold, $\leq$ 5 mm

GRADE B : One or more mucosal breaks > 5 mm confined to folds but not continuous between tops of mucosal folds.

GRADE C : Mucosal breaks continuous between tops of 2 or more mucosal folds but not circumferential.

GRADE D : Circumferential mucosal break.

Helicobacter pylori

Helicobacter pylorus is a spiral shaped micro-organism that has been recognized as the main causal agent of chronic gastritis and duodenal ulcers, and it is associated with the subsequent development of gastric carcinoma. In 1892 – Giulio Bizzozero gave the clear description of spiral bacteria on the gastric mucosa of the Dogs (McFarlane & Munro 1997). In 1896 - Saloon finds spirochetes in the stomachs of cats and mice. In 1906 - First report of spirochetes on the surface of human gastric mucosa in histological slide of gastric carcinoma was reported. In 1921 - Luger discovers spirochetes in the gastric juice, and associates their presence with gastric cancer. In 1924 - Luck and Seth discovers urease in the human stomach, which they believe is naturally occurring. It is now known that one of the virulence factors of H. pylori is the urease enzyme, which splits urea into ammonia. In 1938 – Doenges described spirochetes in the gastric glands of humans and primates (Macacus rhesus). In 1983 – Warren and Marshall described a S – shaped spiral bacteria which was associated with surface epithelial inflammation. In 1983 – The same authors were able to recover these pathogens in culture following prolonged incubation with microaerophillic conditions: it was originally termed as “campylobacter pylori”. In 1984 – Warren and Marshall reported that these bacteria were found in the antrum of

almost patients with chronic active gastritis, duodenal ulcer or gastric ulcer. In 1989 – Closer study of C.Pylori by Goodwin et al showed that C.Pylori possessed a number of chemotaxonomic properties of its own and renamed it as **Heliobacter pylori** based on its spiral shape. In 1993 – National institute for Health consensus conference, Bethesda USA declared infection with Helicobacter pylori is an important cause of duodenal and gastric ulcers. In 1994 – The International Agency for Research on Cancer Classified H.Pylori as a group 1 carcinogen (Julie Parsonnet 1996). In 1997 - Tomb et al. completed sequencing of the entire 1,667,867 base pair H. pylori genome. This assists in identifying new virulence factors for the infectivity of H. pylori on the molecular level. In 2002 - The European Helicobacter Pylori Study Group published the Maastricht 2-2000. In Consensus Report, suggesting a test-and-treat strategy for H. pylori in young patients without atypical symptoms. This strategy advocates the use of non-invasive testing to evaluate for H. pylori and simply treating if found, even in the absence of ulcer disease documented on endoscopy. In 2005 - Warren and Marshall are awarded the Nobel Prize in Physiology / Medicine for their work on H. pylori and Peptic Ulcer Disease. This review into the history of H.Pylori shows that a lot of interest has been shown in studying this small organism in the past years.

H pylori is a curved S-shaped (spiral) flagellated (about 4 – 6) motile, gram negative bacterium. It measures approximately 0.5 microns in width and 2-3 microns in length (Goodwin et al (1985). It has a unique and unusual fatty acid 3 – hydroxycatadecanoic acid on the cell membrane (which is not present in Campylobacters). Therefore it was classified into a separate genus Helicobacter (Lambert et al (1987): Goodwin et al (1989). Motility is darting and rapid due to multiple, sheathed flagella with terminal bulbs and its helical morphology: this facilitates movement through viscous environments such as mucus (Hazell et al

(1986): Lee A et al (1988). It is non-sporulating and grows at 37 degrees C under microaerobic conditions (Megraud F (1989).

The remarkable property of this organism is its ability to split urea, which is not seen in other Helicobacters affecting man (Langerberg et al (1984). When incubated in oxygen on prolonged culture or when exposed to bismuth salts and antibiotics, it transforms morphologically from spiral to coccoid form (Dhawan et al (1997).

Two important mechanisms are urease activity and the presence of sheathed flagella on the outer surface of the bacterium, which enable bacterial transfer through the acidic gastric lumen into the viscous epithelial mucus layer (Suerbaum S. et al 1993). The flagellar filament consists of two different proteins, FlaA and FlaB, which are both essential for motility. (Josenhans C, and Labinge A, et al 1995) Allelic disruption of flaA and flaB, the genes encoding for the FlaA and FlaB proteins, evoked mutant H. pylori strains with reduced motility, that were unable to colonize gnotobiotic piglets (Eaton K, Suerbaum S et al 1996) . Functional studies revaled that motility is dependent on viscosity and pH (Hazell S, Lee A et al 1986).

H. Pylori is a helix-shaped Gram-negative bacterium, about 3 micrometres long with a diameter of about 0.5 micrometre. It is microaerophilic; it requires oxygen although at lower concentration than is found in the atmosphere. It contains a hydrogenase which can be used to obtain energy by oxidizing molecular hydrogen (H_2) that is produced by intestinal bacteria. It produces oxidase, catalase, and urease.

H. Pylori is a spiral or curved micro-aerophilic Gram negative rod, equipped with 4-6 flagellae at one end. H.pylori possesses unipolar, sheathed flagella that, with their spiral shape, allow the organism to move quickly from the lumen of the stomach, where pH is low through the mucus layer to an area where pH is near neutral to

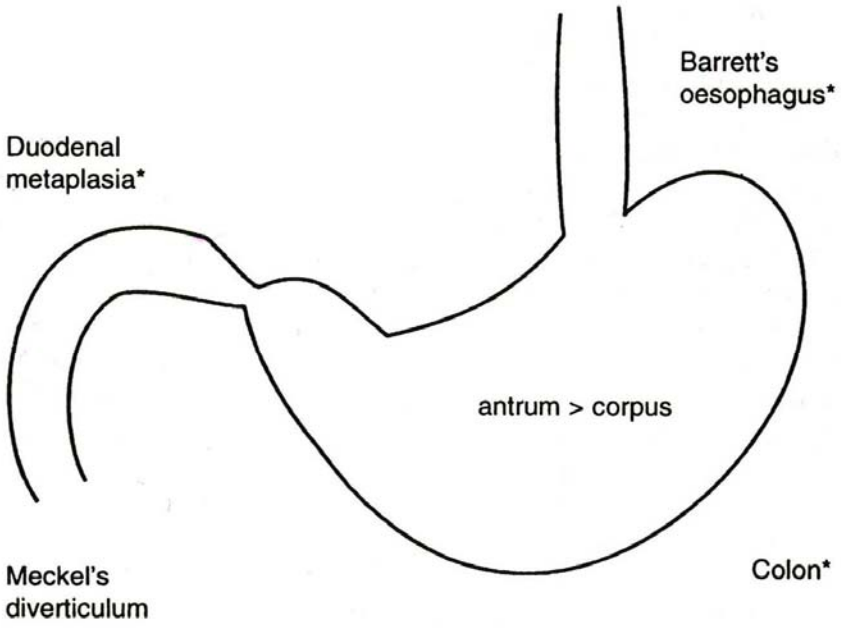
permit optimal growth. H.pylori also prefers the antrum, where parietal cells are absent or scanty. It has the ability to survive in the stomach despite the mucosal immune response. The immune response of the GIT is only successful against bacteria such as H.Pylori, which remain in the lumen. The low pH in the stomach lumen may further impair the effect of IgA antibodies against H.Pylori.

The important feature of H.pylori might be due to the specific binding of H.pylori to the gastric cells of the surface and foveolar type. About 20% bacteria are attached in this way, sometimes through an adhesion pedestal. Narikawa S, and Imai N, (1990).

Upon entering the stomach, H. Pylori heads toward the mucus layer which is rich of high-molecular-weight mucins, urea and sodium bicarbonate. After penetrating this viscous layer, adherence of the bacteria to the nearby epithelium is facilitated by formation of bacterial pedestals (Caselli M and Figura N etal 1989).

The affinity for specific receptor structures presented by different epithelial cells may affect the distribution of H. pylori colonization through out the stomach. Apart from motility and adhesion, survival in the gastric acid environment is facilitated by enzymatic activity of the bacteria. The major enzyme produced by H. pylori is urease. Adhesion may restrict bacterial colonization to the stomach, but may also contribute to tissue damage. H.pylori also colonizes heterotopic or metaplastic gastric epithelium outside the stomach. This includes patches of metaplastic gastric epithelium in the duodenal bulb of patients with duodenal ulcers. Wyatt J, and Rathbone BJ et al (1990). Barrett's epithelium in the oesophagus of the patients with chronic acid reflux. Loffeld RJ, Ten Tije BJ, (1992). Ectopic gastric epithelium in Meckel's diverticula can also be colonized. De cothi GA, Newbold KM, (1989).

SITES OF H.PYLORI INFECTION:



*

INDICATES SITES OF GASTRIC METAPLASIA

Epithelial damage plays a key role in the induction of H.pylori colonization, as it may enable the bacterium to obtain essential nutrients such as iron. It is however also a key factor in the establishment of disease during long – term colonization, and probably also a factor in the prevention of GERD. Epithelial damage is not only the result of ammonia production. Leunk et al, described that supernatants of H. Pylori cultures could induce vacuoles in eukaryotic cells (Leunk RD, Johnson PT etal 1988). Further research revealed that expression of the responsible vacuolating cytotoxin A occurred in only 50% of H. pylori strains even though the gene encoding for this cytotoxin was present in all strains.

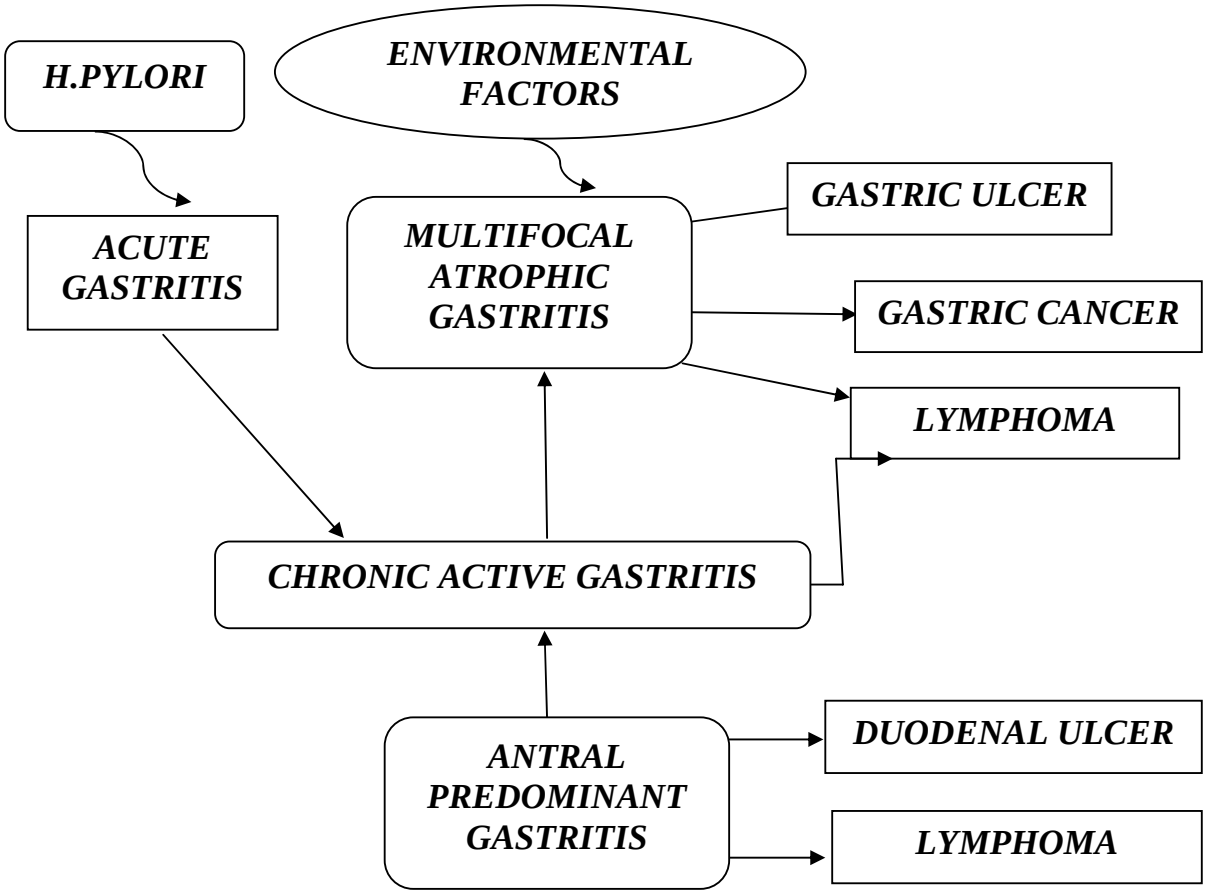
Expression of VacA is considered a marker for more virulent strains with higher cytotoxicity. Different alleles were recently described for the vacA gene, they differ in their middle regions (two variants: m1 and m2) and signal sequence regions (four variants: sla, slb, slc, and S2). Although eight genotypes are thus theoretically possible, strains containing VacA, S2ml have not been observed. Strains containing slml are high toxin producers, whereas s2m2 are low producers. Patients with peptic ulcer disease are more often infected with strains containing the s1 genotype (Atherton JC, Cao P et al 1995).

Strains carrying the CagA+ phenotype thus seem to be more virulent than CagA-strains. Unlike the vacA gene, the cagA gene is not conserved in all H. pylori strains. In developing countries the prevalence of cagA+strains is higher. The cagA gene is 30kb marker for a pathogenicity island, containing several genes including picA and picB (Tummuru MKR, Sharma SA, et al 1995). Transcription of both these genes is linked to that of cagA and lack of transcription of one gene causes lack of

transcription of all downstream genes. Mutations in the cagA gene preserves the virulence of cagA + H. pylori strains, whereas mutations in picA or picB diminish the cytotoxic activity of the strain. The virulence of cagA + strains thus essentially depends on expression of picA and picB.

Strains that contain vacA sl, cagA + or ice Al are considered highly pathogenic and are strongly associated with more severe gastritis and its complications such as peptic ulcer disease, whereas type vacA s2ms, cagA-iceA2 are considered less pathogenic. More virulent strains also have a greater effect on acid production and therefore may play a more significant role in GERD.

PROPOSED NATURAL HISTORY OF H.PYLORI INFECTION



ASSOCIATION OF H. PYLORI WITH BARRETT'S OESOPHAGUS

In a meta – analysis of 26 case – control and cross – sectional studies, several of them being preliminary reports, 562 of 1426 GERD patients were H. pylori positive (39%) compared with 1009 of 2010 control subjects (50%) (O'Connor HJ, 1999).

Reflux disease results from the interaction between acid production, lower esophageal sphincter pressure, esophageal clearance and gastric emptying. H. pylori may affect several of these factors. In particular acid production can be affected in H. pylori – positive subjects by various mechanisms. Some individuals respond to H. pylori colonization with an exaggerated gastrin response, leading to increased acid production and limitation of H. pylori gastritis to the gastric emptying (Gillen D El-Omar EM, et al 1998).

In many others however, gastric acid production is impaired due to several factors, including the release of substances such as the VacA protein, which directly inhibits parietal cell function and bacterial urease activity generating large amounts of acid – buffering ammonia. As a results of these factors, H. pylori gastritis extends into the gastric corpus where mucosal inflammation further impairs acid production, among others by the generation of interleukin-1, which has a 100-fold stronger acid – suppressive capacity than proton pump inhibitors. Most importantly however, more than 50% of the H. pylori positive subjects eventually develop chronic atrophic gastritis. This results in a loss of parietal cells and thus a further impairment of acid production. (Kuipers EJ, Uyterlinde AM, et al 1995) These factors which lead to persistent decrease in acid production can explain why H. pylori may protect against GERD.

THE POSSIBLE ROLE OF HELICOBACTER PYLORI IN GASTROESOPHAGEAL REFLUX DISEASE

A variety of abnormalities contribute to the development of gastroesophageal reflux disease (GERD) including transient lower esophageal sphincter relaxation, low esophageal sphincter pressure, presence of a hiatal hernia, diminished esophageal clearance of refluxed gastric contents, and alterations in esophageal mucosal resistance. Helicobacter pylori infection clearly plays a role in the Pathogenesis of Peptic ulcer disease and mucosa associated lymphoma of the stomach and is a definite risk factor for distal gastric cancer. The role of H. pylori infection in gastroesophageal reflux disease remains controversial and incompletely understood. Although H. pylori infection does not cause reflux disease, circumstantial evidence suggests that it may protect against the development of gastroesophageal reflux disease and its complications in some patients. The most likely mechanism where by H.pylori infection protects against gastroesophageal reflux disease is by decreasing the potency of gastric refluxate in patients with corpus predominant gastritis. (Flak GW, USA DH 44195).

SEVERITY OF GASTROESOPHAGEAL REFLUX DISEASE IN INFECTED AND NON-INFECTED H.PYLORI PATIENTS

It has been postulated that H.pylori infection, if in a body – predominant pattern, would lead to reduced gastric acid, resulting in a reduced acid content in any potential refluxate. In fact, in patients with esophagitis, those not infected with H.pylori had more grade A esophagitis, a finding contrary to the postulate of less severe gastroesophageal reflux disease with infection. Zentilin et al also noted similar comparisons between H.pylori infected and non-infected gastroesophageal reflux disease patients.

The current results are compatible with the findings by Moayeddi et al. In a well –designed study H.pylori infected gastroesophageal reflux disease patients did not experience any increase in relapse of moderate to severe gastroesophageal reflux disease symptoms post-eradication. Tefera et al also found no change in gastroesophageal reflux disease patients 12 weeks after eradication of this infection and infact, schwizer et al found that eradication positively affected gastroesophageal reflux disease relapse.

Bowrey et al examined gastric patterns in gastroesophageal reflux disease patients and found gastritis of the cardia commonly regardless of whether patients were infected or not with H.pylori. However M.Newton et al found that when H.pylori colonised the gastric antrum it was usually found in the gastric fundus. They concluded that H.pylori is not more common and its distribution does not differ in those with esophagitis compared with control subjects and is therefore unlikely to be aetiologically important in these patients. H.pylori however can colonise Barrett's epithelium.

In another study it was concluded that H.pylori colonisation protects against Barretts Esophagus and that the association may be atleast partially mediated through gastroesophageal reflux disease.

In another study the frequency of H.pylori was low in esophageal biopsy specimens (15%) as well as in gastric biopsy specimens (35.6%).

Specialised metaplastic epithelium was not colonised by the bacteria and the presence of H.pylori in the esophagus was always associated with gastric infection. This finding has been reported previously in retrospective studies indicating that H.pylori colonisation of Barrett's mucosa is probably a consequence of gastric infection. Francoual S, Gruppo (1990).

When H.pylori was identified histologically in the gastric fundus or in the esophagus it was always found in the antral biopsy specimens also. This study has shown no difference in the prevalence of H.pylori in patients with esophagitis compared with controls some have reported improvement in reflux after eradication of H.pylori Francoual et al (1990).

Francoual et al suggested that in those with active esophagitis H.pylori was found more commonly in the proximal stomach. Others have not found an association (Johnston DA and Goudie B et al) (1994).

In this study when H.pylori was present it was usually found in both the gastric antrum and gastric fundus, suggesting that H.pylori colonises the whole stomach. (Barthel JS) (1988). though others report a higher frequency of H.pylori in the gastric antrum than the fundus (Bayerdroffer E) (1992).

H.pylori has been shown to colonise the gastric epithelium of Barrett's esophagus. Hazell SL, 1988. Talley NJ 1988.

More recently justin et al, showed that in 30 patients with Barrett's esophagus there was a higher colonisation rate in the metaplastic – esophageal mucosa when esophagitis was also present. Justin TA 1988.

Helicobacter pylori may be found in Barrett's mucosa (Paull G et al 1988) but apparently only when also present in the stomach, thus providing additional evidence for the presence of reflux in this condition.

BARRETT'S OESOPHAGUS (BE):

Norman Barrett first described this entity in 1950 (Spechler SJ 1996). Barrett's esophagus, defined as the presence of columnar metaplastic epithelium in the distal tubular esophagus over a length of more than two to three centimeter, is usually considered a complication of long-standing gastroesophageal reflux disease.

The current definition states that to say BE, both endoscopic and histologic criteria to be met. The endoscopic component requires the presence of columnar mucosa identified endoscopically by its salmon pink colour, extending proximally from the Gastro-esophageal junction into the tubular esophagus. (Spechler SJ 2002). The histologic component requires that the biopsies taken from the endoscopically identified columnar pink mucosa contain metaplastic or intestinalized columnar epithelium with goblet cells (Sampliner RE 2002).

Barrett esophagus is divided into long segment BE (LSBE) in which the columnar mucosa extends 3 cm or more above the GEJ and short segment BE (SSBE), in which the specialized columnar epithelium is restricted to < 2 to 3 cms above the GEJ. BE develops in upto 44% patients with reflux esophagitis. It has a bimodal age distribution with one peak at 0 to 15 years and another at 40 to 80 years.

BE is an acquired metaplastic change that results from long standing GERD. Multi-potential immature stem cells differentiate into various epithelial types, including columnar epithelium which is more resistant to acidic digestion and which is able to regulate more rapidly than the native squamous epithelium.

The development of BE is a multi-step process with atleast 3 distinct phases. During the initiation phase genetically predisposed individuals, suffering from GERD develop reflux esophagitis. This leads to the formation of a metaplastic epithelium with features of intestinal columnar epithelium.

During the formation stage, the metaplastic epithelium, which continues to be exposed to the refluxate, establishes its presence and occupies a variable surface area of the distal esophagus. This results in the oral migration of the squamocolumnar junction over time (Hamilton SR 1977).

A long and multifaceted progressive phase follows, during which the metaplastic epithelium either remains dormant and clinically insignificant or progresses to dysplasia and invasive adenocarcinoma.

Gross and endoscopic features:

BE appears beefy red and velvety, contrasting with the lighter pink coloured smooth squamous mucosa. The Squamocolumnar Junction (SCJ) often lies within 30 cms of the incisor teeth and often co-exits with a hiatal hernia, stricture, diffuse esophagitis or esophageal ulcers.

Patterns of BE:

- ✓ Circumferential, islands and finger-like projections or tongues.
- ✓ Patients with SSBE have short tongues or patches of red mucosa lying < 2 cms above the GEJ

Endoscopy biopsy areas:

1. The stomach just distal to the upper end of the gastric folds, particularly along the lesser curvature.
2. 1 to 2 cm above the GEJ.
3. Tongues of mucosa or irregular areas above the SCJ
4. The SCJ and squamous epithelium of the native esophagus.

Biopsies at the upper end of the gastric folds, may allow one to determine whether there is gastritis, particularly HP induced gastritis and possibly intestinal metaplasia.

Histology of Barrett's esophagus (BE):

The definition of Barrett's esophagus requires histologic confirmation of intestinal metaplasia in biopsies taken from the columnar regions of the esophagus. The metaplastic BE epithelium resembles either small intestinal absorptive cells (complete intestinal metaplasia) or incomplete intestinal metaplasia (resembling colonic epithelium). In the latter, the cells lack a distinct brush border and associated enzymes that normally characterize small intestinal absorptive cells.

The majority of the intestinal columnar cells are so-called intermediate, principal or pseudoabsorptive cells that have characteristics of both absorptive and secretory cells. H.pylori may be found in some of the patients with BE but only when it is also present in the stomach. It may contribute to the severity of the inflammation seen in BE.

Intestinal metaplasia at the Gastro-esophageal Junction (GEJ) is either SSBE, which has a cancer risk at most of 0.5% per year or intestinal metaplasia of the proximal stomach, which appears to have a substantially smaller risk for malignancy. These two conditions cannot be distinguished reliably because the morphologic & histochemical features of gastric & esophageal intestinal metaplasia resemble one another and because the gross landmarks used to identify the GEJ do not have the precision necessary to localize the mucosa.

Squamous metaplasia develops in the distal esophagus following treatment of BE. It appears as a normal appearing neosquamous epithelium or as a multilayered immature squamous metaplasia. The neosquamous epithelium appears in areas previously occupied by BE, often appearing as squamous islands surrounding the Barrett epithelium. Squamous metaplasia resembling that seen in the uterine cervix

develops at the GEJ in patients with BE. It usually appears as a pseudostratified epithelium; cilia are often present on the luminal surface.

DYSPLASIA IN BARRETT'S ESOPHAGUS:

Histopathologically, the development of an adenocarcinoma appears to be preceded by epithelial dysplasia. Often, surrounding an adenocarcinoma in Barrett's esophagus, dysplastic changes can be found. Furthermore, longitudinal follow-up studies have documented the gradually increasing severity of dysplasia eventually resulting in adenocarcinoma. These observations suggest, that dysplastic changes might be taken as early indicators of incipient malignancy.

Dysplasia is defined as neoplastic proflieration within eipithelial glands without affecting the basement membrane. Dysplasia in Barrett's esophagus is classified as low or high grade in fashion comparable to dysplasia in inflammatory bowel disease. (Riddell RH: Goldman et al 1983) This implies that the grade of dysplasia should be determined by the features of the most dysplastic region, either surface or base.

The criteria for the grading of dysplasia in Barrett's esophagus are cited from Haggitt et al (Haggitt RC 1994).

- Low grade dysplasia.

The crypt architecture tends to be preserved and distortion, if present is mild; the nuclei may be stratified, particularly near the base of the crypts, but the stratification does not reach the apical surfaces of the cells; nuclei are enlarged, crowded, and hyperchromatic; mitotic figures may be present in the upper portion of the crypt; and goblet and columnar cell mucus is usually diminished or absent, but goblet cells in which the mucus droplet does not communicate with the surface may be observed. The abnormalities extend to the mucosal surface.

- High grade dysplasia.

Distortion or crypt architecture usually is present and may be marked; it is composed of branching and lateral budding of crypts, a villiform configuration of the mucosal surface, or intraglandular bridging of epithelium to form a cribriform pattern of 'back-to-back' glands, nuclear abnormalities are present as in low grade dysplasia, but stratification reaches the crypt luminal surface, there may be a loss of nuclear polarity, i.e. not perpendicular to the basement membrane, and the nuclei often vary markedly in size, shape, and staining characteristics. Goblet and columnar cell mucus is usually absent. The abnormalities extend to the mucosal surface. In addition, it might be difficult to reliably exclude (micro) invasion within the lamina propria in cases of high grade dysplasia. In fact, beginning microinvasion, defined by an irregular epithelial stromal interface, is considered an integral part of high grade dysplasia in Barrett's esophagus (Cameron AJ: 1997) Another phenomenon that needs attention is the discrimination of reactive changes from true dysplasia. Especially when ulcertation is present one should be cautious in the interpretation of cellular atypia. In these cases it is best to wait for repeat biopsies after adequate anti-reflux therapy. Reactive changes may lead to mild nuclear and cellular atypia, which might be classified as low grade dysplasia. Reactive cytonuclear changes are usually mainly present in the deeper parts of the glands, and do not involve the mucosal surface. This feature can be used to discriminate reactive atypia from true dysplasia. However, in case of doubt the classification 'indefinite for dysplasia' should be applied.

Short – segment Barrett's esophagus:

Intestinal metaplasia at the gastro-esophageal junction has gained great interest recently, since it might be involved in the rapid increase of adenocarcinomas in this region over the past 20 years (Blot WJ et al 1991). The significance of short-

segment Barrett's esophagus (intestinal metaplasia limited to the distal 3 cm of the tubular esophagus), which is increasingly being found both endoscopically and histologically, remains controversial. In most cases it seems to be associated with reflux diease. At present, the risk of developing dysplasia and adenocarcinoma due to short-segment Barrett's esophagus is considered to be low (Weston AP : Krmpotich P et al (1996).

The prevalence of dysplasia appeared two times higher in long – segment Barrett's esophagus, than in short segments of Barrett's esophagus (Hirota WK: Loughney TM et al 1999) Therefore, agreement exists that patients with short segment Barrett's esophagus require surveillance, until long –term follow up studies have clarified its cancer risk.

INTESTINAL METAPLASIA AT THE GASTROESOPHAGEAL JUNCTION

Esophageal adenocarcinoma is strongly correlated with Barrett's esophagus. Cameron et al (1995) found a 100% correlation between esophageal adenocarcinoma and the presence of Barrett's esophagus. The same authors reported that in patients with a junction adenocarcinoma, which was defined as a tumour centered less than 2 cm from the junction, Barrett's esophagus was present in about half of cases. They concluded that adenocarcinomas of the gastro-esophageal junction are associated with short and long segments of Barrett's esophagus. Intestinal metaplasia at the gastro–esophageal Junction was also observed by others (Spechler SJ: Seroogian JM: et al 1994).

Intestinal metaplasia of the gastroesophageal junction is found in approxitamely 10-40% of patients without long segments of Barrett's mucosa (Nandurkar S: Talley NJ: 1997). Spechler et al (1994). Reported that 15-20% of adults undergoing elective upper endoscopy had segments of intestinal metaplasia at

the gastro-esophageal junction, which were not recognized by endoscopy. Trudgill et al (1997), also concluded that intestinal metaplaisa at the junction is a common finding. In general, it is found in the setting of gastritis or related to gastro-esophageal reflux disease. Histologically it may be difficult to discern a short-segment Barrett's esophagus from (focal) intestinal metaplasia of the gastric cardia.

ROLE OF HELICOBACTER PYLORI IN THE METAPLASIA OF (CLO) COLUMNAR-LINED ESOPHAGUS:

There is much interest in the role of Helicobacter pylori (HP) in the pathogenesis of columnar-lined esophagus (CLO) and its progression to adenocarcinoma. There is an increasing body of evidence linking so-called ultra-short segment CLO (USSCLO; now better termed intestinal metaplasia {IM} of the cardia) with HP infection and intestinal metaplasia elsewhere in the stomach (Morales TG, Sampliner RE, 1997). Conversely, there is increasing evidence of a reciprocal relationship between HP and traditional / classical CLO and short segment CLO(Blaser MJ. 1998). It has been suggested that gastric infection, especially pangastric, with cag-A positive strains of HP may be protective against CLO. It may be therefore that the dramatic changes in the prevalence of CLO and esophago-cardiac adenocarcinoma are related to alterations in the prevalence of gastric HP infection. It has been proposed that a crucial determinant of the predominant pathology of the upper gastro-intestinal tract, in evolutionary and epidemiological terms, is the time of acquisition of HP infection. Thus ameliorating socio-economic circumstances and the widespread use of antibiotics may account for a dramatic reduction in HP gastritis in childhood and early adulthood in Western communities over the past few decades. This, in turn, may help to explain the proximal movement in the prevalence of gastric cancer and the increase in GERD, columnar-lined esophagus and esophageal

adenocarcinoma. Perhaps the most important pathogenic factor here is that highly prevalent helicobacter infection (especially cag-A positive HP), often contracted at a young age, leads to pan gastritis and a reduction in acid output from the stomach because of the destructive inflammation within the gastric fundus. The reduction in acid in turn may reduce the propensity to acid-induced reflux esophagitis and thus reduce the incidence of CLO – type metaplasia. Indiscriminate eradication of HP may not be a sensible strategy (at least for esophageal disease) and there must be careful consideration of potential risks as well as benefits of HP eradication policies.

Useful pathological criteria for the diagnosis of dysplasia in columnar-lined esophagus.

Low grade dysplasia consists of mild or moderate adenomatous dysplasia, enlarged, crowded, hyperchromatic and ovoid nuclei. Mitotic activity may be substantial and atypical mitoses may be present Nuclear stratification is often present. Architectural changes, include villosity. There is loss of the basal – luminal maturation / differentiation axis.

High-grade dysplasia consists of severe adenomatous dysplasia . Nuclei are enlarged, usually spheroidal, and have an open chromatin pattern with prominent nucleoli. Mitotic activity may be substantial and atypical mitoses are usually present. Nuclear stratification may be present but there is usually pronounced cellular disorganization. Architectural changes, including villosity, glandular budding and complex glandular structures, is usually present. There is loss of the basal luminal maturation/ differentiation axis.

Patients with BE develop hyperplastic polyps, squamous papillomas, dysplasia and rarely adenomas. The major importance of BE lies in the propensity to develop into an adenocarcinoma.

COMPLICATIONS OF GASTROESOPHAGEAL REFLUX DISEASE:

Increasing age is an important factor in the prevalence of GERD complications. (Collen M J, et al 1995). GERD is associated with considerable morbidity and complications such as esophageal ulceration's, peptic strictures and Barrett's esophagus. Complications peak between the ages of 50 and 70 years (Nebel OT, et al 1976). The severest complication is carcinoma developing in the setting of Barrett's esophagus.

The mucosal changes of reflux esophagitis range from erosions, superficial ulcers, and extension of the inflammatory process, leading to fistula formation.

Erosions are superficial lesions that remain confined to the lamina propria and muscularis mucosae sparing all but the most superficial layers of the submucosa. The necrosis, hemorrhage, and inflammation associated with ulcers, extend deeper into the underlying submucosa or muscularis propria. The epithelium close to erosions or ulcers often contains neutrophils, eosinophils, and many lymphocytes. The erosions or ulcers often contain granulation tissue, an inflammatory exudate, and fibrinoid necrosis in the ulcer base. Lymphoplasmacytic infiltrates, often forming lymphoid aggregates, tend to cluster around erosions and ulcers. Epithelium at the ulcer margin is usually attenuated. Marked basal cell hyperplasia may occupy the entire mucosal thickness and there may be marked acanthosis. These changes may be accompanied by occasional bizarre epithelial or stromal cells.

Erosions or ulcers may be isolated or confluent; they commonly coexist with one another. The damaged mucosa present in reflux esophagitis becomes prone to secondary infections.

If appreciable ulceration has occurred, longitudinal ridges with crests develop. The ridges consist of hyperplastic, hyperkeratotic, acanthotic, squamous epithelium and extensions of lamina propria; the troughs represent linear ulceration. The alternating ridges and ulcers end abruptly at the cardia; they usually taper away gradually into the surrounding squamous mucosa as one proceeds proximally, Pyogenic granulomas may develop.

Esophageal peptic ulcers also develop in the setting of reflux esophagitis; they resemble peptic ulcers occurring elsewhere. These may erode through the muscular layers, resulting in perforation. Peptic ulcers appear large, oval, and well circumscribed with elevated borders and deep necrotic centers. As these heal, strictures develop. This occurs in about 10% of patients with severe reflux esophagitis. Fibrosis is usually present and may extend into the submucosa or beyond, sometimes extending into the periesophageal tissues. Although peptic strictures nearly always involve the distal esophagus, they occasionally develop more proximally. Proximal strictures average 2 to 4 cm in length. Extensive strictures complicate fulminant reflux esophagitis as well as nasogastric intubation in patients with reflux esophagitis or Zollinger –Ellison syndrome.

A large retrospective European study with 6.5 years of follow –up found complications in 21.6% of patients, including 13 patients with esophageal ulcers, 15 with strictures, and 45 patients with Barrett's epithelium (Brossard E et al 1992). However, these data contrasted with other studies in which no patients with erosive esophagitis developed Barrett's esophagus in a 2-year U.S. trial (Spechler SJ 1992) and in which stricture was reported in only 0.26% of 3800 French patients (Rejeb MB 1992) over a 12-year period.

MATERIALS AND METHODS

In our study 150 cases of patients presenting with symptoms of GERD attending medical gastroenterology outpatient department of Tirunelveli Medical College Hospital and a private gastroenterology clinic were included.

The patients were evaluated clinically and subjected to upper Gastrointestinal (GI) endoscopy The findings in the upper gastrointestinal (UG) endoscopy were recorded and biopsy were taken from the esophageal lesions.

Subsequent gastric antral biopsies was also done in these cases during the same sitting The samples were fixed in 10% neutral buffered formalin and processed in routine manner.

4 µ (micron) sections were cut from both the tissues and stained with Haematoxylin and eosin. Gastric biopsy were also stained with Warthins starry stain to evaluate the presence of H. Pylori.

The demographic data were recorded in a Proforma.

The histopathological changes of esophagus were recorded. The findings were correlated with the H. Pylori status of the gastric antral biopsies.

The gastric antral biopsies were stained with haematoxylin and eosin and graded according to the modified sydney system of classification.

STANDARD HEMATOXYLIN AND EOSIN STAIN FOR PARAFFIN SECTIONS

METHOD

1. Dewax sections, hydrate through graded alcohols to water.
2. Remove fixation pigments if necessary.
3. Stain in an alum hematoxylin of choice for a suitable time
4. Wash well in running tap water until sections 'blue' for 5 minutes or less.
5. Differentiate in 1 per cent acid alcohol (1 per cent HCI in 70 per cent alcohol) for 5-10 sec.
6. Wash well in tap water until sections are again 'blue' (10-15 minutes), or
7. Blue by dipping in an alkaline solution (e.g. ammonia water), followed by a 5-min. Tap water wash.
8. Stain in 1 per cent eosin Y for 10 min.
9. Wash in running tap water for 1-5 min.
10. Dehydrate through alcohols, clear and mount.

RESULTS

Nuclei	:	blue/black
Cytoplasm	:	varying shades of pink
Muscle fibers	:	deep pink/red
Red blood cells	:	orange/red
Fibrin	:	deep pink.

WARTHIN – STARRY METHOD FOR SPIROCHETES

(Warthin & Starry 1920)

SECTIONS

Formalin-fixed, paraffin.

SOLUTIONS

Acetate buffer, pH3.6

Sodium acetate	:	4.1 g
Acetic acid	:	6.25 ml
Distilled water	:	500ml

1% silver nitrate in pH 3.6 acetate buffer.

DEVELOPER

Dissolve 3 g of hydroquinone in 10ml pH 3.6 buffer, and mix 1ml of this solution and 15 ml of warmed 5% scotch glue or gelatin; keep at 40^{o}C. Take 3 ml of 2% silver nitrate in pH 3.6 buffer solution and keep at 55^{o}C Mix these two solutions immediately before use.

METHOD

1. Deparaffinize and rehydrate through graded alcohols to distilled water.
2. Celloidinize in 0.5% celloidin, drain and harden in distilled water, 1 min.
3. Impregnate in pre-heated 55-60^{o}C silver solution (b), 90-105 minutes.
4. Prepare and preheat developer in a water bath.
5. Treat with developer (solution c) for $3^{1/2}$ minutes at 55^{o}C. Sections should be golden-brown at this point.

6. Remove from developer and rinse in tap water for several minutes at 55-60^{o}C, then buffer at room temperature.
7. Tone in 0.2% gold chloride.
8. Dehydrate, clear and mount.

RESULTS

Spirochetes : Black

Background : Golden yellow

RESULTS

Table :1

1	Total Number of Cases	150
2	Total Number of Esophageal Biopsy	150
3	Total Number of Gastric Antral Biopsy	138

Table :2

GENDER DISTRIBUTION OF THE CASES

Sl.No	Gender	Number of Cases
1	Male	102 (68%)
2	Female	48(32%)

Table :3

AGE WISE PREVALENCE OF GASTROPHAGEAL REFLUX DISEASE AND GENDER DISTRIBUTION

Sl.No.	Age Group (Years)	No of cases	Male	Female
1	0-10	-	-	-
2	11-20	4	4	-
3	21-30	25	15	10
4	31-40	46	30	16
5	41-50	25	17	8
6	51-60	22	16	6
7	61-70	16	11	5
8	71 & above	12	9	3
	Total	150	102	48

Table : 4

THE DIFFERENT TYPES OF ESOPHAGEAL LESIONS IN THE STUDY

Sl.No.	Type of Lesion	Number of cases
1	Chronic reflux esophagitis	88
2	Barrett's esophagus	46
3	Vascular Ectasia	4
4	Malignancy	12

Table : 5

MALIGNANT LESIONS

Sl.No.	Malignant Lesions	Number of cases
1	Squamous cell carcinoma	9
2	Adenocarcinoma	3

Table : 6

HISTOLOGICAL GRADING OF BARRETT'S OESOPHAGUS

Sl.No.	Histological Grade	Number of cases
1	Classical Barrett's	39
2	Low – Grade Dysplasia	4
3	High- Grade Dysplasia	3

Table : 7

THE CORRELATION OF ESOPHAGEAL LESION (BIOPSY) WITH H.PYLORI STATUS

Sl.No.	Type of Esophageal lesion	Number of cases	Number of cases Positive for H.Pylori
1	Chronic reflux esophagitis	88	34 (39%)
2	Barrett's oesophagus	46	16 (34%)
3	Malignancy	12	0
4	Vascular Ectasia	4	0

Table : 8

VARIOUS GRADES OF BARRETT'S OESOPHAGUS HAVING ASSOCATION WITH H. PYLORI

Sl.no.	Histological grade of Barrett's oesophagus	Number of cases	H.Pylori Positive
1	Classical Barrett's	39	14
2	Low – Grade Dysplasia	4	1
3	High – Grade Dysplasia	3	1

Table : 9

GASTRIC ANTRAL BIOPSIES POSITIVE FOR H. PYLORI

Sl.No.	Biopsy	Number of cases
1	Total number of gastric biopsies	138
2	Number of cases positive for H. Pylori	50 (32.6%)

Table 10 :

THE SEVERITY OF INFLAMMATION IN THE ANTRAL BIOPSIES AND ITS ASSOCATION WITH H. PYLORI

Sl.No.	Severity of Inflammation	Number of cases
1	Mild Inflammation	37 (74%)
2	Moderate Inflammation	10 (20%)
3	Severe Inflammation	3 (6%)

DISCUSSION

In our study we have analysed the histomorphology of esophagus in 150 patients, who presented with symptoms of gastroesophageal reflux disease to the outpatient Department of Medical Gastroenterology and a private Medical Gastroenterology Clinic.

This study carries a unique significance as it was conducted in part of country, where people have different life styles, food and personal habits. Most of the patients were from Tirunelveli and the two neighbouring coastal Districts of Thoothukudi and Kanyakumari.

The study population included both children and adults, however adults were predominant.

We had four children 2.6 (%) and one hundred forty six 97.4. (%)adults. (Wide Table 3.) This correlates with the study of Sonnenberg HB et al (1999), who found a significant rise in the number of children with symptoms of Gastroesophageal reflux Disease. In most of the children in our study, the esophageal changes were that of reflux esophagits and we did not have even a single case of Barrett's. This observation differ from that of Robbins 7th Edition, who found out that reflux esophagits is occasionally seen in infants and children. A much more large sample analysis will help us in to make a definite conclusion.

The peak age groups of patients implicated with symptoms of Gastroesophageal reflux disease were between 32-40 years of age, very much closer to the observation that reflux esophagitis is most common in the middle aged persons Anderson 10th edition. (Wide Table No 3)

The incidence of Barrett's esophagus were found in individuals of fourth or fifth decade and we had very few cases within fourth decade. None of the malignant

cases were seen in younger patients. This is in coherence with the observation of Johnson DA and Fennerty MB (2004) who found out that increasing age is an important factor in the prevalence of Gastroesophageal reflux disease complications and they attritubuted this to the cumulative acid induced injury to the esophagus over time.

The gender distribution of the cases illustrated in Table No:2 We had 102, 68(%) number of male patients and 48 female patients 32(%).

This goes hand in hand with the observation made by various authors, who found that gastroesophageal reflux disease is almost equally seen in both gender, however the esophageal lesions of the gastroesophageal reflux disease were more common in men than in women.

We tried to analyse the esophageal lesions with respect to the gender of the population under study. (Wide table 2). We found out that the men had a higher number of reflux esophagitis and Barrett's esophagus than women. 68(%). This is similar to the observation made by Cecilia M. F who found that there is an unequivocal male predominance of reflux esophagitis and Barrett's esophagus

Regarding the common associated symptoms, most of our cases included in the study presented with heartburn which resembles the observation made by Nebel et al 1976 - 1977 who opined that heartburn is the most common and classical symptoms of Gastroesophageal reflux disease.

In our study, we found that most of the patients expressed heartburn postprandially especially after intake of spicy, fatty food. Few of our cases had this symptom following intake of certain medications for headache and body ache.

Analysis of the esophageal lesions reveal that the most common histomorpholgical changes encountered was that of Chronic reflux esophagitis

58.6(%) followed, by Barrett's 29.3 (%) malignancies 8 (%) and few cases of miscellaneous changes like vascular ecstasias 2.6(%) This correlates well with similar observation made by other authors.

Analysis of the various histomorphological features of esophagus in Gastroesophageal reflux Disease

Epithelial hyperplasia with expansion of the basal zone and elongation of the vascular papillae of the lamina propria was found in most of our cases of Chronic reflux esophagitis. This correlates well with the observation made by Collins BJ et al (1996) who was of the opinion that epithelial hyperplasia indicated a rapid epithelial turnover and proliferation, was a very significant histological change seen in patients with chronic reflux esophagitis. They called this change as a marker of reflux.

Balloon degeneration of the epithelial cells is found to be yet another indicator of epithelial cell injury. The epithelial cells appear swollen, rounded with pale staining cytoplasm.

Jessurun JY etal (1988) proposed that the presence of these balloon cells indicates epithelial cell injury.

We also had many cases, showing balloon degeneration of the squamous epithelium. 42 cases of the total 88 cases Chronic reflux esophagitis showed the presence of balloon cells. This correlates well with the observation of Sternberg vol 2 – V^{th} edition who found that two thirds of the patients with Chronic reflux esophagitis showed presence of balloon cells in the epithelium.

Presence of a markedly dilated lamina propria, with elongated vascular papillae and congested capillaries is yet another finding seen in cases of Gastroesophageal reflux disease, Geboes K etal (1980). Most of our cases of Chronic reflux esophagitis showed elongated vascular papillae with congested lamina propria.

Presence of intra epithelial eosinophils is an additional indicator of Gastroesophageal reflux disease, Tummala V. et al (1987)

Haggi HC (2000) found out that significance could be given to it when more than six eosinophits were present in the specimen. Brown etal (1984) found out, the presence of eosinophils within the epithelium was the most frequent abnormality seen in cases of Chronic reflux esophagitis .

In this present study, only few of our cases with Chronic reflux esophagitis showed presence of eosinophils within the epithelium. In our study 18 cases of total 88 cases showed significant presence of intra epithelial eosinophils.

Barrett's Esophagus

In Barrett's esophagus the squamous cell epithileum has undergone metaplastic chage to columar epithelium, presumably as a result of long standing gastro-esophageal reflux. Spechler SJ and Goyal RK (1986).

The transformed mucosa may have a foveolar, some times a villous pattern with irregular crypts and glands. The metaplastic epithelium mainly consists of columnar cells and goblet cells. Few neuroendorine cells and paneth cells may be present.

In our study, we had 46 patients presenting with Barrett's Esophagus. In most of them, the metaplastic epithelium was made up of columar cells, resembling the gastric mucous cells.

Inflammation was not very significant in the cases of Barrett's Esophagus. We had noticed non-specific inflammation in 7(15%) of our cases and ulceration was seen only in 2 cases 4(%). This is in coherance with the observation made by Petras RE et al (1991), who found that ulceration and inflammation are non-specific changes seen in association with Barrett's esophagus.

Dysplasia and Barrett's esophagus:

Although all patients with Barrett esophagus are at increased risk, certain patients are at higher risk than others. Most Patients with Barrett esophagus – associated adenocarcinoma are older white men (Falk G.W 2002). As previously mentioned, there is also evidence to support the contention that only those patients with goblet cells are at increased risk of developing adenocarcinoma (Hamilton SR et al 1987)

Barrett's esophagus have 30-40 fold increased risk for esophageal adenocarcinoma. The development of an adenocarcinoma appears to be preceded by the presence of epithelial dysplasia. Several longitidunal follow up studies have clearly documented the gradual increase in the severity of dysplasia leads to increased risk of adenocarcinoma. These observations suggest that the dysplastic changes might be taken as early indicator of incipient malignancy.

Dysplasia can be defined as the presence of neoplastic epithelium confined within the basement membrane of the gland from which it arises (Riddell R.H et al 1983)

Dysplasia in Barrett's esophagus is classified into low or high grade in a fashion comparable to the dysplasia in inflammatory bowel disease, Riddell RH et al (1983). This implies that the grade of dysplasia should be determined by the features of the most dysplastic region, either surface or base.

The criteria for grading the dysplasia in Barrett's esophagus has been laid by Haggitt RC (1994). In low grade dysplasia the crypt architecture is preserved with overcrowding of the nuclei near the base of the crypt. Goblet cell numbers are often reduced and dystrophic goblet cells may be present. whereas in high grade dysplasia the distortion of the crypt architecture is marked and is composed of branching

crypts. The epithelium is arranged back to back with loss of nuclear polarity. (Cameron AJ and carpenter HA (1997).

In some cases, the distinction between high – grade dysplasia and intramucosal adenocarcinoma (defined by the penetration of neoplastic cells through the basement membrane to infiltrate into the lamina propria or muscularis mucosae) may be difficult, particularly in a biopsy specimen (Ormsby A.H et al 2002)

Because of its metaplastic nature, the glands at the base of Barrett Mucosa show “baseline atypia” characterized by enlarged, slightly hyper chromatic cells with some stratification and increased mitotic activity. Thus, cytologic atypia involving the surface epithelium is a major diagnostic criterion for making a definitive diagnosis of dysplasia.

In our present study we had 7 cases of dysplasia seen out of 46 cases of Barrett’s esophagus. Of these 4 were typed as low grade dysplasia and 3 as high grade dysplasia, (Table 6).

These cases are being placed under close followup to observe any significant malignant transformation, especially the cases with high grade dysplasia. Weston AP et al (2000) found that about 53% of the patients with high grade dysplasia progressed to multifocal high grade dysplasia or an invasive carcinoma.

In contrast, a large study of patients with Barrett – related high – grade dysplasia suggested that surveillance endoscopy with biopsy is a valid and safe follow – up strategy for patients with high – grade dyplasia without concurrent cancer since only 16% of patients subsequently developed carcinoma during a mean surveillance period of 7.3 years (Schnell TG et al 2001)

Burke et al (1991) was of the opinion that low grade dysplasia is rather indolent and a not a reliable hall mark for malignancy.

GERD and malignancy:

Out of the 150 cases included in our study we found out malignant lesion in 12 cases (8%). The endoscopic picture was classical in 8 of the cases with ulcerated, ulceroproliferative lesion, whereas in the other 4 the endoscopic picture was not classical. Multiple biopsies were taken in suspicion to exclude a malignancy.

Histological analysis of cases of malignancies showed 9 cases (75%) of squamous cell carcinoma and 3 cases (25%) of adenocarcinoma. (Table No 5)

This is comparable to the observation made by Souza R.F (2002), who found that occurrence of squamous cell carcinoma was the most common type of carcinoma followed by adenocarcinoma. Out of the nine cases 7 elderly individuals belong to 5^{th} decade and above. We had 2 cases of esophageal squamous cell carcinoma present in the third decade. Both these patients were found to be chronic alcoholics with a long history of cigratte smoking. They also had histomorphological features of chronic esophagitis in the non malignant area, suggesting the fact that it could have been a trigger for the malignant transformation.

Regarding the site and distribution of squamous cell carcinoma, 5 cases had lesion in the mid esophagus and the rest 4 had a lesion in the lower esophagus. All of them presented with ulcerative type of lesion. Histologically they were categorised between well differentiated squamous cell carcinoma to moderately differentiated squamous cell carcinoma.

In this present study, we had 3 cases of adenocarcinoma of esophagus. Which constitutes 25% of total malignancies seen in our study. In all the three cases there was evidence of Barrett's esophagus with ulceration, in fact in all of them the

endoscopy findings were that of ulcerated Barrett's esophagus. Histologically the diagnosis of adenocarcinoma was made. Two of the three cases had feature of high grade dysplasia in addition to the malignancy and in the other cases we could not find any evidence of dysplasia. This goes hand in hand with the observation made by several workers that majority of adenocarcinoma arise from pre-existing Barrett's Esophagus. (Blot W et al (1993)). In all the three cases, the lesion was located in distal esophagus with infiltration into gastric cardia. Histomorphologically all the three cases were well differentiated adenocarcinoma.

H-pylori and GERD

Another aspect of our study was to determine the prevalence of gastric H.pylori infections in patients with esophageal lesions of GERD. To achieve this, concomitant gastric antral biopsies were taken when the patient was subjected to upper GI endoscopy. Out of the total 150 cases, we were able to obtain gastric, antral biopsy samples for 138 cases. (Wide Table 1)

The gastric antral biopsy was analysed for the type of inflammatory reaction according to modified Sydney system of classification and were broadly grouped into mild, moderate and severe. (Wide Table10)

Warthin starry silver stain was used to detect the presence of H.pylori and it was noted as positive or negative. (Wide Table 9)

The histomorphology of the esophageal lesions were kept blind folded during the evaluation of the gastric antral biopsies to avoid bias in observation.

Out of the total 138 antral biopsies, H.pylori was detected in 50 number of cases (32.6%). H.pylori was not detected in 88(63.7%) number of cases.

The presence of H.pylori status was then compared with that of the esophageal histomorphology.

Chronic reflux esophagits was the most common esophageal lesion studied, and we had 88 cases of the total 150 cases. H.pylori was detected in 34 of the total 88 cases with Chronic reflux esophagitis . (39%). This correlates very well with similar observation made by Abbas Z et al (1995) who found 38% of positivty in their study of 29 cases. They found a equal positivity among patients with Chronic reflux esophagitis and Barrett's Esophagus.

The values of our study is slightly lower than that observed by Weston AP et al (2000), who found 44.2% of their cases with Chronic reflux esophagitis, had gastric H.pylori infection.

Newton M et al (1997) also had a very similar observation like ours. In his study on subjects with GERD he found 36% of the cases of Chronic reflux esophagitis had gastric H.pylori.

Barrett's esophagus was the second common lesion observed in our study. The prevalence of gastric H.pylori was analysed for the patients presenting with Barrett's esophagus.

In our study we found 34% of the patients presenting with Barrett's esophagus had gastric H.pylori infection. This observation matches well with that of Abbas. Z et al loc cit (1995) who found an incidence of 39%. He also analysed the presence of H.pylori in the columnar mucosa of Barrett's esophagus Weston AP et al (2000) had an observation very similar to our study. He was able to demonstrate the presence of H.pylori in 95 of the total 289 cases. (32.9%). He found a positivity of 44.2% in cases of Chronic reflux esophagitis.

Newton M et al and his colleagues in a similar study found about 25% of the cases were positive for H.pylori. Henihan RD et al (1998) came out with a positivity of 23% (19 out of 82 patients) with Barrett's esophagus. He was not able to detect the organism in cases of adenocarcinoma of esophagus.

Gebrud D et al (1998) evaluated the incidence of gastric H.pylori infection in patients with Barrett's esophagus and found that 39% (11 out of 28 cases) had gastric H.pylori infection.

Peitz V et al (1997) had a higher incidence of positivity with 50% of the total cases showing the presence of gastric H.pylori.

A prospective study by Lord RV et al (2000) revealed a gastric H.pylori positivity of 31.3% in cases of Chronic reflux esophagitis and 16.5% in cases of Barrett's esophagus. He was of the opinion that gastric H.pylori infection may have a protective effect for the development of Barrett's esophagus.

The incidence of Helicobacter pylori infection in the patients with Gastroesophageal reflux disease , varies widely in literature from 30% to 90% and approximatemy of 35% in most series .

Various studies have been done to detect the prevalence of gastric H.pylori infection in gastroesophageal reflux disease. In USA , Cheng found out that , of the 27 gastroesophageal reflux disease patients, 41% of them had H. pylori positivity . In his study, he established the presence of H. pylori by means of demonstration of the organism in antral mucosal histology, culture and urease activity. Whereas Abbas in his study done at Pakistan, found it to be higher. He found that 62% of the gastroesophageal reflux disease patients where positive for H. pylori infection . Similarly Liston, who carried out his work at UK also found an increased percentage

of H. pylori positive gastroesophageal reflux disease patients. He fount that up to 76%. of them where infected with H. pylori. In his study, he utilized Serology also to establish the presence of H. pylori . In our study we found that the association was less. We utilized the antral mucosal histology and Warthin starry stain to demonstrate the presence of H. pylori infection. Our study showed that 30% of the gastroesophageal reflux disease patients, were infected with H. Pylori. This correlates well with the various other studies done by others in various parts of the world.

However in another study done by Grande et al (2008), he found no significant evidence for an important role for H. pylori infection in the development of gastroesophageal reflux disease and erosive esophagitis.

Table 11

The following table summarises the result of various studies in the prevalence of gastric H. Pylori in GERD.

Authors	**Geographic area**	**Number of GERD patients**	**% of H.pylori positive**	**Method of establishment of H.Pylori**
Cheng	USA	27	**41%**	Antral mucosal Histology Culture Urease activity
Abbas	Pakistan	29	62%	Antral mucosal Histology
Liston	UK	37	76%	Antral mucosal Histology Urease activity Serology
Werdmuller	Netherlands	118	**29%**	Antral mucosal Histology Urease activity Serology
Newton	UK	36	**36%**	Antral mucosal Histology Urease activity Serology
Varanasi	USA	114	**31%**	Antral mucosal Histology Rapid Urease
Hackelsberger	Germany	130	**39%**	Antral mucosal Histology Rapid Urease Serology
Vicari	USA	84	**36%**	Antral mucosal Histology Serology
Wu	Hongkong	106	**31%**	Antral mucosal Histology Urease activity Serology
Maves	Italy	110	**40%**	Antral corpus Histology Serology
Koike	Japan	175	**34%**	Antral mucosal Histology Corpus Histology Urease activity Serology
Wu	China	225	**34%**	Antral mucosal Histology Corpus Histology Urease activity Serology
Our Study	**India**	**150(50)**	**30%**	Antral mucosal Histology Warthins –starry

Table 12

PREVALENCE OF H.PYLORI IN CASES OF BARRETT'S ESOPHAGUS

The following table summarises the results of various studies on the prevalence of gastric H.Pylori infection in cases of Barrett's esophagus.

Authors	**Geographical area**	**No of cases of Barrett's esophagus**	**% of HP positive**	**Method of establishment of H. Pylori infection**
Paull	USA	26	**39%**	Antral mucosal Histology
Abbas	Pakistan	29	48%	Antral mucosal Histology
Werd Muller	Netherlands	13	23%	Antral mucosal Histology
Csendes	Chile	100	20%	Antral mucosal Histology
Newton	UK	16	25%	Antral mucosal Histology Urease activity
Vicari	USA	48	**31%**	Urease activity
Oberg	Sweden	40	13%	Antral mucosal Histology
Schenk	Netherlands	49	20%	Antral mucosal Histology
Westen	USA	289	**33%**	Antral mucosal Histology
Our study	**India**	**46**	**34%**	Antral mucosal Histology Warthins starry stain

Both GERD and H.pylori colonization are, common phenomenon. Their different geographical distribution is a first hint that H.Pylori may be negatively assocated with GERD.

An analysis of the findings given in the table suggests a lower prevalence of gastric H. Pylori infection in patients with endoscopic signs of GERD.

GERD patients with concomitant H.Pylori infection showed more severe gastritis in the antrum than in other parts of the stomach, such as corpus, fundus and cardia.

Apart from a lower prevalence of GERD among H.Pylori positives, some also reported that if GERD is present in H.Pylori positive subjects it may be less severe .

These observations have been made against the background of changing time trends in the prevalence of H. pylori and the incidence of GERD and GERD complications. As mentioned, the prevalence of H. pylori in Western countries has steadily decreased in the past decades as a result of socio-economic changes. This has led to a significant drop in the incidence of H. pylori-associated disorders such as peptic ulcer disease and adenocarcinoma of the distal stomach. (El-Serag HB et al 1997) In the same period however, the incidence of GERD and GERD complications such as Barrett's esophagus and adenocarcinoma of the gastro-esophageal junction has increased four-to seven-fold (Blaser MJ 1997).

Reflux disease results from intreaction between acid production, lower esophageal sphincter pressure, esophageal clearance and gastric emptying. H. Pylori may affect several of these factors. Few individvals respond to H. Pylori colonization with and exaggerated gastric response leading to increased acid production. These individuals are at the risk of developing duodendal ulcer disease and reflux disease.

Gastric acid production is impaired due to several factors, including the realease of substances such as the VacA protein, which directly inhibits parietal cell function and bacterial urease activity generating large amounts of acid – buffering ammonia. As a result of these factors, H. Pylori gastritis extends into the gastric corpus where mucosal inflammation further impairs acid production, among others by the generation of interleukin – 1, which has a 100-fold stronger acid – suppressive

capacity than proton pump inhibitors. Most importantly however, more than 50% of the H. pylori positive subjects eventually develop chronic atrophic gastritis. This results in a loss of parietal cells and thus a further impairment of acid production. (Kuipers EJ et al 1995). These factors which lead to a persistent decrease in acid production can explain why H. Pylori may protect against GERD.

Therefore epidemiological data strengthen the idea that the H. Pylori protects against GERD developments through the induction of atrophic gastritis.

Schenk etal (1999) observed in a prospective endoscopical study that H. Pylori negative patients had a higher incidence of Barrett's esophgagus than H. Pylori positive GERD patients. We also had a similar observation in our study in which the H. Pylori positivity was lower in Barrett's and it was absent in cases of adenocarcinoma.

Kiltz U et al (2000) in his study concluded that the presence of H. Pylori might delay the development of Barrett's esophagus, which could explain the lower positivity rate of H. Pylori positivity in cases of BE.

SUMMARY & CONCLUSION

Barrett's esophagus is seen in a younger population amongst Indians. A male predominance is noted. There is a paucity of patients with pure dysplasia in Barrett's metaplasia. Despite the fact that there are a number of patients presenting with Barrett's esophagus and carcinoma, very few patients present with dysplasia, indicating that Barrett's esophagus is a silent disease presenting later as a carcinoma.

In summary, the cumulative data support the hypothesis that reduction of acid output by pharmaceutical agents or vagotomy induces and increase of corpus gastritis in H. Pylori positive patients, which leads to further reduction of acid accelerates development of corpus atrophy. Because of the latter phenomenon, H. Pylori eradication has been suggested for younger H. Pylori – positive patients in need of PPI maintenance therapy for GERD. Such a strategy does not seem to have an effect on the efficacy of such therapy, but long-term prospective studies have to show that it prevents the development of atrophic gastritis.

H. Pylori colonization is very common among humans, with individual strains showing clear variations in pathogenetic properties all H. Pylori positive subjects have chronic active gastritis. H. Pylori colonization and associated gastritis strongly interact with gastric acid production.

The evidence is accumulating that in particular the latter pattern of H. Pylori colonization protects against GERD. This hypothesis is supported by the finding of a low H. Pylori prevalence, in particular of the cagA- positive type, in GERD patients and patients with GERD complications.

H. pylori not only plays a role in the etiology of GERD, but also in the treatment of GERD.

The presence of H. Pylori corpus gastritits augments the effects of acid suppressive medication, in particular of proton pump inhibitors. This may have some effect during the initial treatment, where some suggest that H. Pylori positive patients show a somewhat quicker healing and symptom reduction than H. Pylori – negative patients. During maintenance therapy however, H. Pylori has little effect on the efficacy of Proton-pumb inhibitors (PPI) therapy and H. Pylori – positive and negative patients require similar does of PPI. However, in this phase the ongoing profound acid suppression facilitates the persistent presence of a more prominent chronic active body gastritis. This accelerates the progression to gland loss or atrophic gastritis. This effect can be prevented by H. pylori eradication in those who need maintenance PPI treatment for GERD. Although the long – term consequences of this phenomenon still have to be elucidated further H. Pylori eradication should currently be considered in younger H. pylori – positive GERD patients requiring maintenance PPI therapy.

Master Chart

SL.No.	Biopsy No	Name	Age	Sex	Esophageal Biopsy	Antral Biopsy	H.Pylori status
1	1832	K.R. Subramanian	71	M	Barrett's esophagus	Mild Inflammation	Positive
2	1830	R. Kausalya	36	F	Barrett's esophagus	Mild Inflammation	Positive
3	1826	Natarajan	35	M	Barrett's esophagus	Mild Inflammation	negative
4	1851	Nataraja Palani	36	M	Barrett's esophagus	Mild Inflammation	negative
5	1903	Master Amith Raj	12	M	Barrett's esophagus	Mild Inflammation	negative
6	1952	Antony Ganana Henson	25	M	Chronic reflux esophagitis	Mild Inflammation	Positive
7	1812	Murugan	34	M	Barrett's esophagus	Mild Inflammation	Positive
8	1791	Prem	34	M	Barrett's esophagus	Mild Inflammation	negative
9	1789	Angeline	49	F	Chronic reflux esophagitis	Mild Inflammation	Positive
10	1775	Chellammal	58	F	Chronic reflux esophagitis	Moderate Inflammation	Positive
11	1773	Subramanian	22	M	Chronic reflux esophagitis	Mild Inflammation	negative
12	1741	Andiappan	33	M	Barrett's esophagus	Mild Inflammation	negative
13	1739	Kalayana Sundaram	40	M	Barrett's esophagus	Mild Inflammation	Positive
14	1680	Fathimuthu	65	F	Barrett's esophagus	Mild Inflammation	Positive
15	1677	Suganthi	57	F	Chronic reflux esophagitis	Mild Inflammation	Positive
16	1654	Livingston	47	M	Barrett's esophagus	Mild Inflammation	Positive
17	1652	Muthukrishnan	48	M	Chronic reflux esophagitis	Mild Inflammation	Negative
18	1593	Marthan	63	M	Chronic reflux esophagitis	Mild Inflammation	Positive

19	1591	Rajmohan	47	M	Chronic reflux esophagitis	Mild Inflammation	Negative
20	1589	Sivasethuramalingam	74	M	Well differentiated squamous cell carcinoma	Mild Inflammation	Negative
21	1630	Maheshwaran	35	M	Barrett's esophagus	Mild Inflammation	Positive
22	1628	Razool Nisha	44	F	Chronic reflux esophagitis	Mild Inflammation	Negative
23	1625	Absarkhan	40	M	Barrett's esophagus	Mild Inflammation	negative
24	1559	Abdul Kayam	34	M	Barrett's esophagus	Mild Inflammation	Positive
25.	1534	SeethaLakshmi	32	F	Barrett's esophagus	Mild Inflammation	Positive
26	1532	Ramesh	37	M	Chronic reflux esophagitis	Mild Inflammation	negative
27	1397	Vinodh	20	M	Chronic reflux esophagitis	Mild Inflammation	negative
28	1395	Murugesan	48	M	Barrett's esophagus	Mild Inflammation	Positive
29	1402	Gangadharan	37	M	Chronic reflux esophagitis	Mild Inflammation	negative
30	1400	Muruganandham	47	M	Barrett's esophagus	Mild Inflammation	Positive
31	1756	Raja Ramalingam	74	M	Barrett's esophagus	Mild Inflammation	Positive
32	1745	Radhakrishnan	58	M	Barrett's esophagus	Mild Inflammation	Positive
33.	2201	MuthuBala	25	M	Chronic reflux esophagitis	Mild Inflammation	Negative
34.	2732	Murugan	40	M	Barrett's esophagus	Mild Inflammation	negative
35	2691	Mohammed Subair	26	M	Chronic reflux esophagitis	Mild Inflammation	negative
36	5978	Ganesh Babu	31	M	Chronic reflux esophagitis	Mild Inflammation	negative
37	5889	Gomathy	39	F	Chronic reflux esophagitis	Mild Inflammation	negative
38	6512	Thilagavathy	31	F	Barrett's esophagus	Mild Inflammation	negative

39	6489	Kamalakannan	30	M	Barrett’s esophagus	Mild Inflammation	Positive
40	6467	Subbiah Thevar	63	M	Chronic reflux esophagitis	Mild Inflammation	Positive
41	3891	Mohammed Isaque	37	M	Barrett’s esophagus	Mild Inflammation	Positive
42	7980	Pandian	24	M	Chronic reflux esophagitis	Mild Inflammation	negative
43	2663	Biswas	38	M	Chronic reflux esophagitis	Mild Inflammation	Positive
44	2187	Sumaitha Beevi	33	F	Vascular Ectasia	Mild Inflammation	Negative
45	2663	Chidambaranthan	22	M	Chronic reflux esophagitis	Mild Inflammation	Negative
46	2267	Mariappan	38	M	Chronic reflux esophagitis	Mild Inflammation	Positive
47	2637	Appasamy	32	M	Barrett’s esophagus	Mild Inflammation	Positive
48	2515	Chandrasekaran	36	M	Barrett’s esophagus	Mild Inflammation	Negative
49	2332	Abdul Hussain	29	M	Chronic reflux esophagitis	Mild Inflammation	Negative
50	2335	Mohammed Aneez	18	M	Barrett’s esophagus	Mild Inflammation	Negative
51	2334	Saravanan	28	M	Barrett’s esophagus	Mild Inflammation	Negative
52	2245	Padamsena	66	M	Chronic reflux esophagitis	Mild Inflammation	Positive
53	2201	Mithunsekar	25	M	Chronic reflux esophagitis	Mild Inflammation	Positive
54	2162	Shenbagavalli	27	F	Chronic reflux esophagitis	Mild Inflammation	Positive
55	2140	Senthilkumar	31	M	Chronic reflux esophagitis	Mild Inflammation	Positive
56	2090	Kanchanadevi	42	F	Chronic reflux esophagitis	Mild Inflammation	Positive
57	2092	Ganesan	39	M	Chronic reflux esophagitis	Mild Inflammation	Positive
58	2094	Chandrasekar	49	M	Barrett’s esophagus	Mild Inflammation	Positive
59	2096	Mohan	34	M	Barrett’s esophagus	Mild Inflammation	Positive

60	2042	Chelladurai	29	M	Chronic reflux esophagitis	Mild Inflammation	Negative
61	2020	Muthukrishnan	29	M	Barrett's esophagus	Mild Inflammation	Negative
62.	2022	Jaikannan	20	M	Vascularectasia	Mild Inflammation	Negative
63.	2001	Gopalakrishan	50	M	Chronic reflux esophagitis	Moderate Inflammation	Negative
64	2003	Rangasamy	53	M	Barrett's esophagus	Moderate Inflammation	Positive
65	1989	Ramanarayanan	80	M	Poorly differentiated squamous cell carcinoma	Mild Inflammation	Negative
66	3421	Kothai	54	F	Barrett's esophagus	Moderate Inflammation	Positive
67	3424	Baskar	37	M	Squamous cell carcinoma	Moderate Inflammation	Negative
68	3428	Vijayan	22	M	Barrett's esophagus	Mild Inflammation	Negative
69	3404	Susiladevi	37	F	Chronic reflux esophagitis	Mild Inflammation	Negative
70	3408	Prabhukannan	27	M	Barrett's esophagus	Mild Inflammation	Negative
71	3410	Sabiral	48	F	Barrett's esophagus	Mild Inflammation	Negative
72	3412	Marimuthu	34	M	Chronic reflux esophagitis	Moderate Inflammation	Negative
73	3366	Fairose Begum	35	F	Barrett's esophagus	Mild Inflammation	Negative
74	3326	Irshad	35	M	Chronic reflux esophagitis	Mild Inflammation	Negative
75	3221	Niyaz	25	M	Chronic reflux esophagitis	Mild Inflammation	Negative
76	3223	Manikandan	26	M	Barrett's esophagus	Mild Inflammation	Positive
77	3226	Basheer	40	M	Chronic reflux esophagitis	Mild Inflammation	Positive
78	3230	Gothandapandi	41	M	Barrett's esophagus	Mild Inflammation	Negative
79	3166	Muthulakshmi	27	F	Chronic reflux esophagitis	Mild Inflammation	Negative

80	3168	Jegadesan	42	M	Barrett’s esophagus	Mild Inflammation	Positive
81	2975	Soundarakumari	58	F	Chronic reflux esophagitis	Mild Inflammation	Negative
82	2978	Albet	49	M	Barrett’s esophagus	Mild Inflammation	Positive
83	3129	Ramakrishan	28	M	Vascular Ectasia	Mild Inflammation	Negative
84	3130	Thirumalaikumar	28	M	Barrett’s esophagus	Mild Inflammation	Positive
85	3066	Selvarajan	67	M	Chronic reflux esophagitis	Mild Inflammation	Positive
86	3069	Latha	40	F	Barrett’s esophagus	Mild Inflammation	Negative
87	3030	Thangam	59	F	Chronic reflux esophagitis	Mild Inflammation	Negative
88	3034	Maharajan	27	M	Barrett’s esophagus	Moderate Inflammation	Negative
89	2837	Muthupandi	36	M	Adenocarcinoma	Mild Inflammation	Negative
90	2839	Murugan	46	M	Chronic reflux esophagitis	Mild Inflammation	Positive
91	2843	Michael	22	M	Barrett’s esophagus	Mild Inflammation	Negative
92	2739	Vellathai	35	F	Vascular ectasia	Mild Inflammation	Negative
93	1828	Pannerselvan	34	M	Chronic reflux esophagitis	Mild Inflammation	Negative
94	1827	Balasubramanian	40	M	Chronic reflux esophagitis	Mild Inflammation	Positive
95	1853	Subbulakshmi	34	F	Chronic reflux esophagitis	Mild Inflammation	Negative
96	1852	Shahul Hameed	41	M	Chronic reflux esophagitis	Mild Inflammation	Negative
97	1901	Irduaya Felo	71	M	Squamous cell carcinoma	Mild Inflammation	Negative
98	1900	Majeed	72	M	Adeno carcinoma	Mild Inflammation	Negative
99	1898	Ranjitham	45	F	Chronic reflux esophagitis	Mild Inflammation	Negative
100	1897	Janaki	75	F	Squamous cell carcinoma	Mild Inflammation	Negative

101	1922	N. Prasad	14	M	Chronic reflux esophagitis	Mild Inflammation	Negative
102	1920	B. Roy	30	M	Chronic reflux esophagitis	Mild Inflammation	Negative
103	1954	Maridurai	43	M	Chronic reflux esophagitis	Mild Inflammation	Negative
104	1953	Kumarasamy	49	M	Chronic reflux esophagitis	Mild Inflammation	Negative
105	1815	ArulRani	37	F	Chronic reflux esophagitis	Mild Inflammation	Negative
106	1814	Sankari	18	F	Chronic reflux esophagitis	Mild Inflammation	Negative
107	1813	Annadurai	75	M	Adenocarcinoma	Mild Inflammation	Negative
108	1810	Banu	38	F	Chronic reflux esophagitis	Mild Inflammation	Negative
109	1792	Seetha	35	F	Chronic reflux esophagitis	Moderate Inflammation	Negative
110	1776	Esakki	35	M	Chronic reflux esophagitis	Severe Inflammation	Negative
111	1737	Sulochana	58	F	Chronic reflux esophagitis	Mild Inflammation	Negative
112	1678	Abdul Wahab	25	M	Chronic reflux esophagitis	Mild Inflammation	Negative
113	1655	Babu	28	M	Chronic reflux esophagitis	Mild Inflammation	Positive
114	1594	Rajalakshmi	67	F	Chronic reflux esophagitis	Severe Inflammation	Negative
115	1627	Velusamy	38	M	Barrett’s esophagus	Moderate Inflammation	Negative
116	1561	Ranjit	38	M	Barrett’s esophagus	Mild Inflammation	Positive
117	1560	Noorul Ameer	56	M	Chronic reflux esophagitis	Severe Inflammation	Negative
118	1551	Dhakshina Moorthy	77	M	Squamous cell carcinoma	Mild Inflammation	Negative
119	1556	Thirumalaikumar	34	M	Chronic reflux esophagitis	Mild Inflammation	Negative
120	1555	Petchiammal	38	F	Chronic reflux esophagitis	Mild Inflammation	Negative

121	1453	Fathemia Remosa	28	F	Chronic reflux esophagitis	Mild Inflammation	Negative
122	1452	Antonyammal	54	F	Chronic reflux esophagitis	Moderate Inflammation	Negative
123	1451	Hajarmuthu	52	M	Chronic reflux esophagitis	Mild Inflammation	Negative
124	1450	Mookammal	47	F	Chronic reflux esophagitis	Mild Inflammation	Negative
125	1535	Fathima	27	F	Chronic reflux esophagitis	Mild Inflammation	Negative
126	1530	Vijayakumari	31	F	Chronic reflux esophagitis	Mild Inflammation	Positive
127	1495	Mohammed	66	M	Squamous cell carcinoma	Mild Inflammation	Negative
128	1494	Pitchaiah	47	M	Chronic reflux esophagitis	Mild Inflammation	Negative
129	1492	Leelavathy	46	F	Chronic reflux esophagitis	Mild Inflammation	Negative
130	1491	Vasantha	48	F	Chronic reflux esophagitis	Severe Inflammation	Negative
131	1379	Radha	29	F	Chronic reflux esophagitis	Mild Inflammation	Negative
132	1378	Thiruvaranga Selvi	34	F	Chronic reflux esophagitis	Mild Inflammation	Positive
133	1377	Manikandan	28	M	Chronic reflux esophagitis	Mild Inflammation	Negative
134	1398	Viondh	25	M	Chronic reflux esophagitis	Mild Inflammation	Negative
135	1394	Pattu Navoraj	51	F	Chronic reflux esophagitis	Mild Inflammation	Negative
136	1114	Muthu Ramalingam	57	M	Chronic reflux esophagitis	Mild Inflammation	Negative
137	2181	Sunitha Beevi	33	F	Chronic reflux esophagitis	Mild Inflammation	Negative
138	2171	Habina Ummara	73	F	Squamous cell carcinoma	Mild Inflammation	Negative
139	2751	Sivagnanam	34	M	Chronic reflux esophagitis	Mild Inflammation	Negative
140	2741	Ganesapandi	36	M	Chronic reflux esophagitis	Mild Inflammation	Negative
141	2701	Mohan	49	M	Chronic reflux esophagitis	Mild Inflammation	Negative

142	3001	Barani	44	F	Chronic reflux esophagitis	Mild Inflammation	Positive
143	2951	Alagamuthu	39	M	Chronic reflux esophagitis	Mild Inflammation	Positive
144	2941	Subaidhar	69	M	Squamous cell carcinoma	Mild Inflammation	Negative
145	2931	Ajay	25	M	Chronic reflux esophagitis	Mild Inflammation	Positive
146	2921	Jayasekaran	46	M	Chronic reflux esophagitis	Mild Inflammation	Negative
147	6531	Barakath Fathima	39	F	Chronic reflux esophagitis	Mild Inflammation	Negative
148	2446	Soosan	49	F	Chronic reflux esophagitis	Mild Inflammation	Negative
149	2412	Sabeena	45	F	Chronic reflux esophagitis	Mild Inflammation	Negative
150	2367	Pavithra	25	F	Chronic reflux esophagitis	Mild Inflammation	Positive

BIBLIOGRAPHY

1. Atherton JC, Cao P, Peek RM, Tummuru MKR, Blaser MJ, Cover TL, Mosaicism in vacuolating cytotoxin alleles of Helicobacter pylori J Biol Chem, 1995; 270:17771-17777.
2. Att wood SEA, DeMeester TR, Brenner CG, et al. Alkaline gastro esophageal reflux: Implications in the development of complications in Barrett's columnar lined esophagus. Surgery 106: 764, 1989.
3. Attwood SEA, Ball CS, Jenkinson L, Norris TL, Watson A. Role of intragastric and intraeosophageal alkalinisation in the genesis of complications in Barrett's columnar lined lower oesophagus. Gut 1993;34:11-5.
4. Biancani P, Billett G, Hillemeier et al. Acute experimental esophagitis impairs signal transduction in cat lower esophageal sphincter circular muscle. Gastro entorology 1992; 103: 1199.
5. Blaser MJ, Kobayashi K.Cover TL, Cao P, Feurer ID, Perez-Perez Gl. Helicobacter pylori infection in Japanese patients with adenocarcinoma of the stomach. Int J Cancer, 1993;55:799-802.
6. Blaser MJ, Perez-Perez GI, Kleanthous H, Cover TL, Peek RM, Chyou PH, Stemmerman GN, Nomura A. Infection with Helicobacter pylori strains possessing cagA is associated with an increased risk of developing adenocarcinoma of the stomach Cancer Res, 1995;55: 2111-2115.
7. Blaser MJ. All Helicobacters are not created equal-should all be eliminated? Lancet, 1997; 349 1020-1022.

8. Blot WJ: Devesa SS:Kneller RW: Fraumeni JF. Rising incidence of adenocarcinoma of the esophagus and gastric cardia. JAMA 1991;265:1287-9.
9. Boixeda D, Gisbert JP, Canton R, Baleriola LA, Sanz E, De Argila CM. Is there any association between, Helicobacter pylori infection and reflux esophagitis? Gut 1995: 37; A 57.
10. Bremner RM, Crookes PF, DeMeester TR, Peters JH, Stein HJ, Concentration of refluxed acid and esophageal mucosal injury Am J Surg 1992; 164:522-7.
11. Burke AP, Sobin LH:Shekitka KM:Helwig EB. Dysplasia of the stomach and Barrett esophagus: A follow-up study. Mod pathol 1991;4:336-41.
12. Burner Apsobin, LH shekitka, KM Helwig Dysplasia of the stomach and Barrett's esophagus: A follow up study Mod pathol 1991; 4: 336 -41.
13. Button BM, Roberts S, Kotsimbos TC, et al Gastro esophageal reflux (symptomatic and silent) a potentially significant problem in patients with cystic fibrosis before and after lung transplantation. J heart lung transplant 2005; 24 : 1522.
14. Cameron AJ, Lomboy CT. Barrett's esophagus: Age, prevalence, and extent of columnar epithelium. Gastroenterology 1992; 103: 1241-5.
15. Cameron AJ: Carpenter HA. Barrett's esophagus: High grade dysplasia and early adenocarcinoma: A pathological study. AM J Gastroenterol 1997; 92: 586-91.
16. Cameron AJ:Lomboy CT:Pera M:Carpenter HA. Adenocarcinoma of the esophagogastric junction and Barrett's esophagus. Gastroenterology 1995;109.1541-6.

17. Cameron ZJ:Carpenter HA. Barrett's esophagus:high-grade dysplasia and early adenocarcinoma: A pathological study. Am J Gastroenterol 1997;92:586-91.
18. Caselli M. Figura N, Trevisani L, Pazzi P, Guglielmetti P, Bovolenta MR, Stabellini G. Patterns of Physical modes of contact between Campylobacter pylori and gastric epithelium:implications about the bacterial pathogenicity. Am J Gastroenterol, 1989; 84:511-513.
19. Cecilia m. Fenoglio – preiser Gastro Intestinal pathology an Atlas and text. Third edition Lippincott, Williams, Wilkins.
20. Chen YY:Antonioli DA:Spechler SJ:Zeroogian JM:Goyal RK:Wang HH.Gastroesophageal reflux disease versus Hellcobacter pylori infection as the cause of gastric carditis. Mod Pathol 1998;11:950-6.
21. Clark GWB:Smyrk TC:Burdiles P:Hoeft SF:Peters JH:Kiyabu M:Hinder RA:Bremner CG:DeMeester TR. Is Barrett's metaplasia the source of adenocarcinomas of the cardia? Arch Surg 1994:129:609-14.
22. Collins BJ, Elliott. H, Sloan JM, et al. Oesophageal Histology in reflux esophagitis J. Clin Pathol 1985.
23. Cover TL, Dooley CP, Blaser MJ, Characterization of and human serologic response to proteins in Helicobacter pylori broth culture supernatants with vacuolizing cytotoxin activity Infact Immun, 1990. 58:603-610.
24. Cover TL, Glupczynski Y, Lage AP, Burette A, Tummuru MK, Perez-Perez GI, Blaser MJ. Serologic detection of infection with cagA+Helicobacter pylori strains. J Clin Microbiol, 1995:33:1496-1500.

25. Cover TL, Tummuru MKR, Cao P, Thompson SA, Blaser MJ. Divergence of genetic sequences of the vacuolating cytotoxin among Helicobacter pylori strains. J Biol Chem. 1994; 269: 10566-10573.
26. Crabtree JE, Wyatt JI, Sobala GM, Miller G, Tompkins DS, Primrose JN, Morgan A. Systemic and mucosal humaoral responses to Helicobacter pylori in gatric cancer. Gut, 1993; 34:1339-1343.
27. De koster E, Ferhat M, Deprez C, Delteure M. Helicobacter pylori, gastric histology, and gastroesophageal reflux disease. Gastro entrology 1995: 108:A81.
28. Deltenre M, De Koster E, Ferhat M, Deprez C. Helicobacter pylori, gastric histology and gastroesophageal reflux disease. Endoscopy 1995, 27, 525.
29. Doods WJ, Dent J, Hogan W, Arndorfer R: Effect of atropine on esophageal motor function in humans. Am J physiol 241: G290, 1981.
30. Eaton K,Suerbaum S, Josenhans C, al e. Colonization of gnotobiotic piglets by Helicobacter pylori deficient in two flagellin genes. Infect Immun, 1996; 64:2445-2458.
31. El-Serag HB:Sonnenberg A:Jamal MM:Kunkel D:Crooks L:Feddersen RM. Characteristics of intestinal metaplasia in the gastric cardia. Am J Gastroenterol 1999;94:622-7.
32. Fahmy N. King JF. Barrett’s esophagus: An acquired condition with genetic predisposition. Am J Gastroenterol 1993; 88:1262-5.
33. Fass J, Silny J, Braun J, Heindrichs U, Dreuw B, Schumpelick V et al. Measuring esophageal motility with a new intraluminal impedance device. Scand J Gastroenterol 1994; 29;693-702.

34. Fiorucci S, Santucci L, Chiucchiu S, et al. Gastric acidity and gastroesophageal reflux patterns in patients with esophagitis. Gastroenterology 1992; 103: 855.
35. Francoual S, Lamy Ph, Le Quintrec Y, Luboinski J, petit JC. Helicobacter Pylori: has it a part in the lesion of the gastroesophageal reflux? J infect Dis 1990; 162: 1414.
36. Geboes K; Desmet V. Vantrappen G et al. Vasular changes in the esophageal mucosa. An early histologic sign of esophagitis gastrointestinal Endosc 1980.
37. Goldblum JR:Vicari JJ:Falk: GW:Rate TW:Peek RM:Easly K:Richter JE. Inflammation and intestinal metaplasia of the gastric cardia. The role of gastroesophageal reflux and H.pylori infection. Gastroenterology 1998;114:633-9.
38. Grande et al; licensee BioMed Central Ltd., World Journal of Surgical Oncology 2008, 6:74doi: 10.1186/1477-7819-6-74.
39. Gruppo Operatino Per Lo studio Delle Precancerosi Esofagee (GOSPE): Heli C Bact Pylori in Barrett's esophagus. Histopathology 1991 18:568-70.
40. Haggitt RC. Barrett's esophagus, dysplasia and adenocarcinoma. Hum Pathol 1994; 25; 982-93.
41. Haggitt RC. Histo pathology of reflux induced esophageal and supraesophageal injuries Am J med 2000.
42. Hameeteman W. Tytgat GNJ:Houthoff HJ:Van Den Tweel JG. Barrett's esophagus: Development of dysplasia and adenocarcinoma. Gastroenterology 1989;96:1249-56.
43. Hamilton SR, Yardley JH: Regeneration of cardiac type mucosa and acquisition-of Barrett's mucosa after esophagogastrostomy. Gastroenterology 1977;72;669.

44. Hassall E. Barrett's esophagus: Congenital or acquired? Am J Gastroenterol 1993;88:819-24.
45. Hazell S, Lee A. Brady L, al e. Campylobacter pyloridis and gastritis: association with intercellular spaces and adaptation to an environment of mucus as important factors in colonization of the gastric epithelium, J Infect Dis, 1986; 153:658-663.
46. Helm JF, Dodos WJ, Pek LR, et al. effect of esophageal emptying and saliva on clearance of acid from the esophagus N. Engl J med 310: 284, 1984.
47. Helm JF, Doods WJ, Hogan WJ et al. Acid neutralizing capacity of human saliva. Gastroenterology 83: 69, 1987.
48. Hirota WK: Loughney TM: Lazas DJ Mayodonontch CL:Rholl; V:wong RK specialised intestinal metaplasia; dysplasia and cancer of the esophagus and esophagogastric junction: prevalence and clinical data. Gastroenterology 1999; 116: 277-85.
49. Hollowarth ME, smith M, Kvietys PR, et al. Esophageal blood flow in the cat: Normal distribution and effects of acid perfusion. Gastroenterology 90: 622, 1986.
50. Holloway RH, Kocyan P, Dent J: Provocation of transient lower esophageal sphincter relaxation by meals in patients with symptomatic gastroesophageal reflux. Dig dis Scin 36: 1034, 1991.
51. Holloway RH, Penagini R, Ireland Criteria for objective definition of transient lower esophageal sphincter relaxation. Am J physiol 269: G 128, 1995.

52. Hopkins RJ, Girardi LS, Turney EA. Relationship between anti-Helicobacter pylori eradication and reduced duodenal and gastric ulcer recurrence. Gastroenterology, 1996; 110: 1244-1252.
53. Jessurum J. Yardley JH, Giardiello FM, et al. Intracytoplasmic plasma proteins in distended esophageal squamous cells (balloon cells) Mod pathol 1988.
54. Johnston DA, Fennerty MB Heart burn Severity under estimates erosive esophagitis severity in elderly patients with gastro esophageal reflux disease. Gastroenterology 126: 660, 2004.
55. Johnston DA, Gondie B, Anderson JT, Hopwood D, Pennington CR. A Posible role for Helocobacter pylori in the pathogenesis of refluex esophagitis? World congresses of Gastroentrology. Abstracts 1994; A 1907.
56. Josenhans C, Labigne A, Suerbaum S. Comparative ultrastructural and functional studies of Helicobacter pylori and Helicobacter mustulae flagellin mutants:both flagellin subunits, FlaA and FlaB, are necessary for full motility in Helicobacter species. J Bacteriol, 1995; 175; 3278-3288.
57. Kahirlas PJ, Dodos WJ, Hogan WJ, et al. Esophageal peristaltic dysfunction in peptic esophagitis. Gastroenterology 91: 897, 1986.
58. Karim Q, Dhir N, Baron J, al e. Urease influences Helicobacter pylori motility at the gastric mucosal surface. Gut, 1997, 37:A19.
59. Korstein MA, Rosman AS, Fishbein S, etal: chronic Xerostomia increases esophageal acid exposure and is associated with esophageal injury. Am J med 90: 701, 1991.

60. Kuipers EJ, Perez-Perez GI, Meuwissen SGM, Blaser MJ. Helicobacter pylori and atrophic gastritis; importance of the cagA status. J Natl Cancer Inst, 1995; 87:1777-1780.
61. Kuipers EJ, Uyterlinde AM, PeOa AS, Roosendaal R. Pals G.Nelis GF, Festen HPM, Meuwissen SGM. Long term sequelae of Helicobacter pylori gastritis. Lancet, 1995; 345:1525-1528.
62. Levine DS:Haggitt RC:Blount PL Rabinovitch PS:Rusch VW:Reid BJ. An endoscopic biopsy protocol can differentiate high-grade dysplasia from early adenocarcinoma in Barrett's esophagus. Gastroenterology 1993; 105:40-50.
63. Lieberman meffert D, Alogower M, schimd P, Blum AL., Muscular equivalent of the lower esophageal sphincter gastroenterology 76:31, 1979.
64. Marshall BJ, Warren JR, Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet 1984, 1311-5.
65. Meyers RL, Orlando RC: In Vivo bicarbonate secretion by human esophagus. Gastroenterology 103: 1174, 1992,.
66. Mihara H.,Haruma K, Kamada K, Klyohira K, Low prevalence of H.Pylori infection in patients with refluex esophagitis Gut 1996.
67. Miller , Vinayek R, Frucht H, et al. Reflux esophagitis in patients with ZollingerEllison syndrome Gastroenterology 1990; 98 : 341.
68. Miros M:Kerlin P:Walker N. Only patients with dysplasia progress to adenocarcinoma in Barrett's esophagus. Gut 1991;32:1441-6.
69. Mittal RK, Holloway RH, Penagini R, et al. Transient lower esophageal sphincter relaxation. Gastroenterology 109: 601, 1995,

70. Mittal RK, MC Callum RW. Characteristic of transient lower esophageal sphincter relaxation in humans. Am J physiol 252: G 636, 1987.
71. MittalRK, Lange Rc, Mc Callum RW, Identification and mechanism of delayed esophageal acid clearance in subjects with hiatus hermia. Gastroenterology 92: 130, 1987.
72. Morales TG:Sampliner RE:Bhattacharyya A. Intestinal metaplasia of the gastric cardia. Am J Gastroenterol 1997;92:414-8.
73. Nandurkar S:Talley NJ:Martin CJ:Ng TH:Adams S. Short segment Barrett's esophagus: Prevalence: Diagnosis and associations. Gut 1997;40:710-5.
74. Nebel OT, Fornes MF, Castell DO: symptomatic gastroesophageal reflux incidence and precipitating factors. Dig Dis Sci 1976; 21 : 953.
75. O'connor HJ. Helicobacter pylori and gastroesophageal reflux disease – clinical implications and management. Aliment pharmacol Ther, 1999.
76. Orlando RC, Bryson JC, Powell, DW: Mechanism of H^+ injury in rabbit esophageal epithelium. Am J physiol 246: G 718, 1984.
77. Orlando RC: Esophageal epithelial defenses against acid injury. Am J Gastroenterology 89: 349, 1994.
78. Ormsby AH, Petras RE, Henricks WH, et al – observer variation in the diagnosis of superficial esophageal adenocarcinoma. Gut 2002 ; 51:671-676.
79. Ormsby AH:Goldblum JR:Rice TW:Richter JE:Falk GW:Vaezi MF:Gramlich TL. Cytokeratin subsets can reliably distinguish Barrett's esophagus from intestinal metaplasia of the stomach. Hum Pathol 1999;30:288-94.

80. Orr WC, Robinson MG, Johnson LF etal: Acid clearance during sleep in the pathogenesis of reflux esophagitis Dig Dis Sci 26: 423, 1987.
81. Pandofino JE, Shi G, True Worthy B, Kahrilas, PJ: Esophagogastric junction opening during relaxation distinguishes non-hernia reflux patients, hernia patients and normal subjects gastroenterology 125:1018, 2003.
82. Paull G. Yardley JH Gastric and esophageal campylobacter pylori in patients with Barrett's esophagus. Gastroenterology 1988; 95:216 -218.
83. Peek RM, Miller GG, Tham KT, Perez-Perez GI, Zhan XM, Atherton JC, Blaser MJ, Heightened inflammatory response and cytokine expression in vivo to cagA+Helicobacter pylori strains. Lab Invest, 1995: 73:760-770.
84. Peek RM, Thompson SA, Donahue JP, Tham KT, Atherton JC, Blaser MJ, Miller GG, Adherence to gastric epithelial cells induces expression of a novel ulcer-associated H.pylori gene, iceA, that is associated with clinical outcome. Proc Assoc Am Physicians, 1998; 100:531-544.
85. Pellegrini CA, Demeester TR, Wernly JA, et al. Alkaline gastro esophageal reflux. Am J surg 75: 177, 1978.
86. Pera M. Trastek VF, Pairolero PC, Cardesa A, Allen MS, Deschamps C. Barrett's Disease: Pathophysiology of metaplasia and adenocarcinoma. Ann Thorac Surg 1993;56:1191-7.
87. Pereira AD: suspiro A. Chaves. PSaraiva A: Gloria L:De Almeida JC:Leitao CN: Soares J:Mira FC.Short Segments of Barrett's epithelium and intestinal metaplasia in normal appearing oesophagogastric junctions: The same or two different entities? Gut 1998:42:659-62.

88. Powell J. McConkey CC. The rising trend in esophageal adenocarcinoma and gastric cardia. Eur J cancer prev 1992; 1:265-9.
89. Quddus MR, Henley JD, Sulaiman RA, Palumbo. TC, grepp DR. Helicobacter pylori infection and adenocarcinoma arising in Barrett's esophagus. Hum pathol 1997; 28: 1007-1009.
90. Rauws EAJ, Tytgat GNJ. Cure of duodenal ulcer associated with eradication of Helicobacter pylori Lancet, 1990;335: 1233-1235.
91. Reid BJ, Blount PH, Rubin CE, et al. Predictors of progression to malignancy in Barrett's esophagus: endoscopic, histologic and flow cytometric followup of a cohort. Gastroenterology 1992.
92. Riccardi VM, Rotter Jl. Familial Helicobacter pylori infection. Societal factors, human genetics, and bacterial genetics. Ann Intern Med, 1994; 120:1043-1045.
93. Riddell RH:Goldman H.Ransohoff DF:Appelman HD:Fenoglio CM:Haggitt RC:Ahren C.Correa P:Hamilton SR:Morson BC:Sommers SC:Yardley JH.Dysplasia in inflammatory bowel disease: Standardized classification with provisional clinical application. Hum pathol 1983;14:931-66.
94. Robbins and Cotran 7th edition Elsevier.
95. Sampliner RE: Practice Parameters Committee of the American College of Gastroenterology. Updated guidelines for the diagnosis, surveillance, and therapy of Barrett's esophagus. Am J Gastroenterol 2002;97:1888.
96. Schenk BE, Kurpers EJ, Klinkenberg-knol EC, Eskes SA, Meuwissen SGM. Helicobacter Pylori and the efficacy of omeprazole for gastroesophageal reflux disease. AMJ Gastroenterol 1999.

97. Schhell TG, Sontag SJ, Chief fec G, etal Longterm non-surgical management of Barrett's esophagus with high – grade dysplasia – Gastroenterology 2001; 120: 1607.
98. Sharma SA, Tummuru MKR, Miller GG, Blaser MJ Interleukin-8 response of gastric epithelial cell lines to Helicobacter pylori. Infect Immun, 1995; 63: 1681-1687.
99. Sleisenger and Fordtrans Gastrointestinal and liver disease 8th edition Elsevier.
100. Sloan S, Rademaker AW, Kahirlas PJ. Determinants of gastro esophageal junction incompetence -Hiatal Hermia, lower esophageal sphincter or both? Ann Intern Med 117 : 977, 1992.
101. Sonnenberg A, EL serag HB: clinical epidemiology and natural history of gastroesophageal reflux disease – Yale J Biol Med 1999 ; 12;81
102. Spechler SJ, Goyal RK, Barrett's esophagus N.Engl J Med 1986.
103. Spechler SJ: Barrett's esophagus and esophageal adenocarcinoma: pathogenesis, diagnosis, and therapy. Med Clin North Am 2002;86:1423.
104. Spechler SJ:Zeroogian JM: Antonioli JA:Wang HH:Goyal RK Prevalence of metaplasia at the gastro-esophageal Junction Lancet 1994;344;1533-6.
105. Sternberg's Diagnostic Surgical pathology volume 2. Jaypee, Litincott, Williams and Wilkins.
106. Suerbaum S. Josenhans C.Labigne A. Cloning and genetic characterization of the Helicobacter pylori and Helicobacter mustelae flaB flagellin genes and construction of H.pylori flaA- and flaB-negative mutants by electroporation-mediated allelic exchange. J Bacteriol, 1993; 15: 3278-3288.

107. Talk GW. Barrett's esophagus-Gastro entearology 2002:122:1569-1591.

108. Torrado J, Ruiz B, Garay J, Asenjo JL, Tovar JA, cosme A, correa P. Blood group phenotypes, sulfomucins and Helicobacter pylori in Barrett's esophagus AM J surg pathol 1997, 21: 1023-1029.

109. Trudgill NJ:Suvarna SK:kapur KC:Riley SA. Intestinal metaplasia at the squamocolumnar junction in patients attending for diagnostic gastroscopy. Gut 1997;41:585-9.

110. Tummala V, Barwick KW, sontag SJetal. The significance of intra epithelial eosniophils in the hisologic diagnosis of gastroesophageal reflux. Am J clin Pathol 1987.

111. Tummuru MKR, Sharma SA, Blaser MJ.Helicobacter cagA, a homolog of the Bordutella pertussis toxin secretion protein, is required for induction of IL-8 in gastric epithelial cells. Mol Microbiol, 1995; 18: 867-876.

112. Vaezi MF, Richter JE. Synergism of acid and duodenogastro-esophageal reflux in complicated Barrett's esophagus. Surgery 1995; 117: 699-704.

113. Varanasi RV, Fantry GT, wilson KT. Decreased prevalence of Helicobacter pylori infection in gastroesophageal reflux disease Helicobacter 1998.

114. Werdmuller BFM, Loffeld RJLF. Helicobacter pylori has no role in the pathogenensis of Reflux esophagitis . Dig Dis sci 1997.

115. Weston AP, Badr AS, Topalovski M, cherian R, Dixon A, Hassanein RS. Prospective evaluation of the prevalence of gastric Helicobacter pylori infection in patients with GERD, Barrett's esophagus, Barrett's dysplasia, and Barrett's adenocarcinoma. AM J Gastroenterol, 2000.

116. Weston AP, sharma P, Topalovski M, et al. Longterin follow up of Barrett's high grade dysplasia. AM J Gastroenterol 2000.

117. Weston AP: krmpotich P:Makdisi WF: Cherian R. Dixon A:Mc Gregor DH: Banerjee SK. short segment Barrett's esophagus: clinical and histologic features associated endoscopic findings and association with gastric intestinal metaplasia. AM J Gastroenterol 1996: 91, 981-6.

118. Wijinhoven BPL:Siersema PD:Hop WCJ:van Dekken H:Tilanus HW. Adenocarcinomas of the distal esophagus and gastric cardia are of the distal esophagus and gastric cardia are of the clinical entity. Br J Surg 1999;86:529-35.

119. Winters C, spurling TJ, Chobanian SJ, Curtis DJ, Esposito RL, Hacker JF et al. Barrett's Esophagus. A prevalent, occult complication of gastro-esophageal reflux disease. Gastroenterology 1987;92:118-24.

120. Witman T. Feler A, Rosztoczy A, Jarmay K, Lenart ZS.Does the Helicobacter pylori infection aggravate gastroesophagal reflux disease? Gut 1995:37:A224.

121. Worku M, Sidebotham R, Baron J, Misiewicz J, Logan R, Keshavarz T, Karim G. Motility of Helicobacter pylori in a viscous environment. Eur J Gastroenterol Hepatol 1999; 11: 1143-1150.

122. Wright TA, Myskow M, Kingsnorth AN. Helicobacter Pylori colonization of Barrett's esophagus and its progression to cancer Dis esophagus 1997.